PB84-233287

New and Revised Chemical Fate Test Guidelines
October 1984

(U.S.) Environmental Protection Agency
Washington, DC

Oct 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-233287

CG-1410
October, 1984

PARTITION COEFFICIENT (n-OCTANOL/WATER) ESTIMATION BY LIQUID CHROMATOGRAPHY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

50272-101

REPORT DOCUMENTATION PAGE

1. REPORT NO.
EPA 560/6-84-003

2.

3. Recipient's Accession No.
PB84 233287

4. Title and Subtitle
New and Revised Chemical Fate Test Guidelines

5. Report Date

6.

7. Author(s)

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
Office of Pesticides and Toxic Substances
Office of Toxic Substances (TS-792)
United States Environmental Protection Agency
401 M St., S.W.
Washington, D.C. 20460

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G)

12. Sponsoring Organization Name and Address
Same as box 9.

13. Type of Report & Period Covered
Annual Report

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

These documents constitute a set of 2 new chemical fate test guidelines and support documents that will be added to the chemical fate test guidelines and support documents that had been published by NTIS in October, 1982. There is also a major revision of one test guideline and support document and minor revisions of 4 test guidelines and support documents that had appeared in the October, 1982 publication. These revisions were made in response to public comments.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement
Release Unlimited

19. Security Class (This Report)
Unclassified

20. Security Class (This Page)
Unclassified

21. No. of Pages
322

22. Price

(See ANSI-Z39.18)

See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

CHEMICAL FATE TESTING GUIDELINES
TABLE OF CONTENTS

Guideline/Support Document Title	Guideline No.	Support Document No.	Most Recent Date of Issue
PHYSICAL AND CHEMICAL PROPERTIES			
Absorption in Aqueous Solution: ultraviolet/visible spectra	CG-1050		1982
Boiling Temperature	CG-1100		1982
Density/Relative Density	CG-1150		1982
Dissociation Constants in Water	CG-1200		1982
Henry's Law Constant	CG-1250		1982
Melting Temperature	CG-1300		1982
Particle Size Distribution/Fiber Length and Diameter Distributions	CG-1350		1982
Partition Coefficient (n-Octanol/Water)	CG-1400		1983
Partition Coefficient (n-Octanol/Water)-Estimation by Liquid Chromatography	CG-1410	CS-1410	1984
pH of Water Solution or Suspension	CG-1450		1982
Water Solubility	CG-1500	CS-1500	1983
Water Solubility (Generator Column Method)	CG-1510	CS-1510	1983
Vapor Pressure	CG-1600	CS-1600	1982
TRANSPORT PROCESSES			
Soil Thin-Layer Chromatography	CG-1700	CS-1700	1983
Sediment and Soil Adsorption Isotherm	CG-1710	CS-1710	1983

TRANSFORMATION PROCESSES

Biodegradation, Aerobic Aquatic	CG-2000	CS-2000	1982
Biodegradation, Ready	CG-2010		1982
Biodegradation, Anaerobic	CG-2050	CS-2050	1982
Biodegradation in Soil	CG-2075		1982
Biodegradation, Sewage Treatment Simulations	CG-2100		1982
Complex Formation Ability in Water	CG-4000		1982
Hydrolysis as a Function of pH at 25°C	CG-5000	CS-5000	1982
Photolysis in Aqueous Solution in Sunlight	CG-6000	CS-6000	1984
Laboratory Determination of the Direct Photolysis Reaction Quantum Yield in the Laboratory and sunlight Photolysis	CG-6010	CS-6010	1984
Gas Phase Absorption Spectra and Photolysis	CG-7000	CS-7000	1983

Contents

Page

I. INTRODUCTION.................................... 1

A. Background and Purpose....................... 1
B. Definitions.................................. 3
C. Principle of the Test Method................. 4
D. Applicability and Specificity............... 5

II. TEST PROCEDURES................................ 6

A. Test Conditions.............................. 6

1. Special Laboratory Equipment............ 6
2. Purity of Solvents and Reagents......... 6

B. Preparation of Reagents and Solutions....... 7

1. Solvents................................ 7
2. Calibration Mixture..................... 7
3. Test Solution........................... 9

C. Performance of the Test..................... 9

III. DATA AND REPORTING............................ 10

A. Test Report.................................. 10
B. Specific Analytical Procedures.............. 10

IV. REFERENCES..................................... 11

DISCLAIMER

Certain commercial equipment, instruments, and materials are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the Environmental Protection Agency, nor does it imply that the material, instruments, or equipment identified are necessarily the best available for the purpose.

I. INTRODUCTION

A. Background and Purpose

Since the pioneering work of Hansch and Fujita (1964) in the measurement and estimation of the octanol/water partition coefficient (K_{ow}), this property has become the cornerstone of a myriad of structure-activity relationships (SAR). Hansch and Leo (1979) have used the coefficient extensively for correlating structural changes in drugs with changes observed in biological, biochemical or toxic effects. These correlations are then used to predict the effect of a new drug for which a K_{ow} could be measured.

In the study of the environmental fate of organic chemicals, the coefficient has become a key parameter. It has been shown to be correlated to water solubility, soil/sediment adsorption coefficient, and bioconcentration. The importance of this property to SAR is indicated by its discussion in the first chapter of Lyman, Reehl and Rosenblatt's (1982) comprehensive compendium of methods for estimating the behavior of organic compounds in the environment. These authors consider the measurement or estimation of the coefficient to be the necessary first step in assessing the fate of new chemicals.

Of the three properties that can be estimated from K_{ow}, water solubility is the most important because it affects both the fate and transport of chemicals. For example, highly soluble chemicals become quickly distributed by the hydrologic cycle, have low adsorption coefficients for soils and sediments, and tend to be more easily degraded by microorganisms. In addition, chemical transformation processes such as hydrolysis and oxidation tend to occur more readily if a compound is soluble.

Direct correlations between K_{ow} and both the soil/sediment adsorption coefficient and the bioconcentration factor are to be expected. In these cases compounds that are more soluble in octanol (more hydrophobic) would be expected to partition out of the water and onto the organic portion of soils/sediments and into lipophilic tissue. The relationship between K_{ow} and the bioconcentration factor, as developed by Neely et al. (1974), and other similar relationships, are the principal means of estimating bioconcentration factors. These factors are then used to predict the potential for a chemical to accumulate in living tissue. As a rough estimate, chemicals with K_{ow} less than 10 will not accumulate in tissue while those with K_{ow} greater than 10^6 will. Thus, although a chemical may be present in the aqueous environment at subtoxic concentrations, if its K_{ow} is greater than 10^6 it would accumulate to levels that may be toxic not only to the organism but also to the consumers of that organism.

This test guideline describes a rapid, inexpensive method based on reverse phase-high pressure liquid chromatography (RP-HPLC) for estimating the octanol/water partition coefficient as developed by Veith et al. (1979). It is not intended, however, to replace the standard shake-flask method described in Test

Guideline CG-1400, and should be used keeping in mind the limitations described herein. The RP-HPLC method is intended to give quick estimates of K_{ow}, particularly for very hydrophobic substances and mixtures that cannot be analyzed using the shake flake method.

B. Definitions

(1) The octanol/water partition coefficient (K_{ow}), as estimated by this test method, is the ratio of the equilibrium molar concentrations of a chemical in n-octanol and water, in dilute solution; as such it is a dimensionless quantity. K_{ow} is a constant for a given chemical at a given temperature. Because K_{ow} can assume such a wide range of values, from less than one to greater than a million, depending on the structure of the compound, K_{ow} is often reported log K_{ow}.

(2) The retention time, t_R, is the time in minutes elapsed between sample injection into the chromatograph and the peak maximum (concentration) as recorded on a chromatogram. The retention time is characteristic of the substance, the liquid phase flow rate, and the stationary phase, at a given temperature. With proper flow and temperature control, it can be reproduced to within one percent and used to identify multiple peaks. Although several substances can have nearly identical retention times, each substance has only one retention time. This retention time is not influenced by the presence of other components. Retention times for this method vary between several

minutes for substances with a lower K_{ow} to thirty minutes or greater for substances with higher K_{ow}'s.

C. Principle of the Test Method

This test method is based on a reverse-phase high pressure chromatographic (HPLC) separation procedure developed by Veith et al. (1979). The test substance (solute) is injected onto an HPLC column containing a support onto which a long-chain hydrocarbon has been permanently bonded. A methanol/water solvent system is used to elute the solute which is subsequently analyzed using an ultraviolet absorption detector, gas chromatograph, liquid scintillator or other suitable detector. During elution, the solute moves alone the column by partitioning between the mobile phase and the stationary hydrocarbon phase. The retention time on the column is a function of the hydrophobicity of the solute: A water soluble solute has a short retention time while a hydrophobic solute has a long retention time. Once the retention time is measured on the chromatogram, the K_{ow} of the substance is estimated from a previosly established linear regression equation between log t_R and log K_{ow}. The relationship between these two variables is determined through a calibration step that involves injecting into the chromatograph a mixture of six reference chemicals having a range of retention times and known octanol/water partition coefficients. The retention time for each chemical is measured and a plot of log t_R vs. log K_{ow} is made. The data are also correlated using a linear regression and the resulting equation is used to calculate log K_{ow} from the log

t_R of test substances; the correlation coefficient of the linear regression gives a measure of the "goodness of fit" of the calibration data to a straight line.

D. Applicability and Specificity

The test method described in this guideline is designed to calculate an estimated value of the octanol/water partition coefficient using an empirically derived equation that relates the K_{ow} of a substance to its experimentally determined retention time on a HPLC column. It must be emphasized that the shake flask method (CG-1400) remains the conventional method for determining K_{ow}. The HPLC method described herein is a rapid procedure for estimating log K_{ow} for a single substance or a mixture of substances. Estimates of log K_{ow} should be limited to within two log units of the minimum and maximum values of the calibration substances, i.e., the method is applicable to substances with log K_{ow} between zero and eight. In the range of two to six log units, estimates are within 22.5 ± 20.1 percent of the values reported in the literature obtained using other methods (Veith et al. 1979).

II. TEST PROCEDURES

A. Test Conditions

1. Special Laboratory Equipment

(1) A liquid chromatograph equipped with a 6000 psi pump, a high-pressure stopflow injector, and appropriate recorder.

(2) A preparative scale reverse phase column (250 mm X 8 mm I.D.), e.g., Varian Preparative Micropak C-H, consisting of a stainless steel tube filled with 10 micron LiChrosorb to which octadecylsilane is permanently bonded.

(3) For chemicals that absorb in the ultraviolet (i.e., aromatics), either 254-nm fixed wavelength detector or 190 to 600 nm variable wavelength detector, can be used. For chemicals that cannot be detected in the ultraviolet, a fraction collector can be used to collect fractions at suitable intervals (0.50 to 1.0-minutes near the retention time) for analysis by gas chromatography, liquid scintillation, or other suitable, sensitive, analytical detector.

2. Purity of Solvents and Reagents

All solvents (water, methanol, acetone, and cyclohexane) and reagents used in this test procedure should be reagent or HPLC grade and contain no impurities that could interfere with the determination of the retention time of the test compound. Water meeting ASTM Type II standards or an equivalent grade is recommended to minimize the effects of dissolved salts and other impurities. ASTM Type II water is described in ASTM D1193-77, "Standard Specification for Reagent Water".

B. Preparation of Reagents and Solutions

1. Solvents

For column elution and preparation of buffers, mix chromatographic or reagent grade methanol and water in an 85:15 v/v ratio.

2. Calibration Mixture

Prepare a standard calibration solution containing 200 mg/L of each of the substances listed in Table 1 dissolved in acetone and cyclohexane (3:1 v/v) other suitable solvent. Twenty microliters of this solution injected into the chromatograph should give an adequate recorder response (25% of scale) for calibration purposes. However, both the concentration and amount injected may be increased or decreased without affecting the retention times, since t_R is independent of concentration in dilute solutions.

Table 1. Measured octanol/water partition coefficients and typical HPLC retention times for the chemicals used in the calibration mixture.

Chemical	Literature log K_{ow}	Measured Typical Retention Time minutes	log minutes
Benzene	2.13	4.12	0.61
Bromobenzene	2.99	7.09	0.85
Biphenyl	3.76	8.85	0.95
Bibenzyl	4.81	15.87	1.20
p,p'-DDE	5.69	21.98	1.34
2,4,5,2',5'-PCB	6.11	31.58	1.50

3. Test Solution

Solutions of the test substance(s) are prepared similar to the calibration mixture: by dissolving the substance(s) to be tested in a 3:1 mixture of acetone and cyclohexane. The concentration of the substance(s), as determined by trial and error, should be sufficient to produce a chromatographic peak of at least 25 percent of the recorder scale.

C. Performance of the Test

After conditioning the column with 85:15 methanol-water or buffered methanol-water, chromatograph the calibration mixture by injecting 20 microliters of the mixture into the column. Elute the column using a solvent flow rate of about 2.0 ml/min at a pressure of approximately 1200 psi. Determine the retention time for each substance in the mixture. The calibration mixture must be chromatographed daily because the retention time is sensitive to variations in the flow rate, temperature, solvent ratio, and the retention properties of the column.

Chromatograph 20 microliters of the test solution(s) directly following column calibration, using an identical flow rate and pump pressure. Determine the retention time(s) for the test substance(s).

III. DATA AND REPORTING

A. Test Report

Using the measured retention times of the substances in the calibration mixture and the log K_{ow} for each substance (Table 1) make a plot of log t_R vs. log K_{ow}. From the data used in making this plot compute a linear regression equation of the form:

$$\log K_{ow} = m \log t_R + b$$

i.e., $y = mx + b$. For each set of test conditions (flow rate, pressure) report this equation, its correlation coefficient and the data used in its calculation.

Calculate an estimated log K_{ow} for each test substance from its retention time and corresponding regression equation. Report the retention time, its logarithm and log K_{ow} along with the above data.

B. Specific Analytical Procedures

(1) Provide a detailed description of, or reference for, the liquid chromatograph, separation column, and detector.

(2) Report the temperature at which the test(s) were conducted.

(3) Give a description of any problems (and their rectification) or changes in the test procedures.

IV. REFERENCES

Hansch C, Fujita T. 1964. - - analysis. A method for the correlation of biological activity and chemical structure. J Am Chem Soc 86:1616.

Hansch C, Leo A. 1979. Substituent constants for correlation analysis in chemistry and biology. J. Wiley & Sons. New York.

Lyman WJ, Reehl WF, Rosenblatt DH. 1982. Handbook of Chemical Property Estimation Methods: Environmental Behavior of Organic Compounds. McGraw-Hill Book Company. New York.

Neely WB, Branson DR, Blau GE. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ Sci Technol 8:113.

Veith GD, Austin NM, Morris RT. 1979. A rapid method for estimating log P for organic chemicals. Water Res 13:43.

CS-1410
October, 1984

PARTITION COEFFICIENT (n-OCTANOL/WATER) ESTIMATION BY LIQUID CHROMATOGRAPHY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Table of Contents

Page

I. NEED FOR THE TEST 1

II. SCIENTIFIC ASPECTS 3

- A. Rationale For the Test Method 3
- B. Other Methods For Determining K_{ow} 4
  - 1. The Conventional Shake-Flask Method 4
  - 2. Estimation from Water Solubility 5
  - 3. Estimation Using the Fragment Constant Method........................ 7
  - 4. Estimation Using Thin-Layer Chromatography 8
- C. Rationale for the Test Conditions 9
  - 1. Chromatographic Column 9
  - 2. Analytical Detector 9
  - 3. Purity of Solvents and Reagents........ 9
  - 4. Calibration Mixture 10
- D. Test Data and Reporting 10
  - 1. Test Report............................ 10
  - 2. Analytical Procedures.................. 11

III. REFERENCES 12

I. NEED FOR THE TEST

The octanol/water partition coefficient (K_{ow}) is one of the most frequently measured and most widely used parameters in assessing the environmental fate of organic chemicals. Its importance is due primarily to the fact that K_{ow} is used to predict the general lipophilicity or hydrophobicity of a chemical. Of greater significance on the quantitative level, however, is the fact that K_{ow} has been found to correlate with the water solubility, the soil/sediment adsorption coefficient and the bioconcentration factor.

Although all of the above mentioned properties are important in predicting the fate and residence time of a chemical in the environment, the bioconcentration factor (BCF) carries with it the greatest consequences. This is because the BCF is used to predict the potential for a chemical to accumulate in living tissue. When applied to the aquatic environment, the BCF is indicative of the potential for a chemical to accumulate or bioconcentrate in the tissue of fish and other lower aquatic organisms. In terms of the K_{ow}, chemicals with a value less than 10 will not bioconcentrate while those with a value greater than 10^4 will. Thus, although a chemical may be present in a stream or lake at subtoxic concentrations, if its K_{ow} is greater than 10^4 it could accumulate to levels that may be toxic to not only the organism itself, but also to the consumers of that organism, not the least important of which are Homo sapiens.

The direct determination of K_{ow}, using the conventional shake-flask method (CS-1400) is subject to numerous experimental difficulties. These include the formation of colloidal dispersions (emulsions) during the shaking step, incomplete separation of the octanol and water phases, difficulty in analyzing very hydrophobic chemicals in the water phase, the adsorption of the solute onto the surfaces of transfer vessels, and the loss of volatile solute into the atmosphere. In addition the method is time consuming and the chemicals used in the test procedure, including the test substance, must be of high purity. Some of the principal advantages of the HPLC method are: 1) the test substance need not be pure and, in fact, may actually contain any number of impurities or other test substances: 2) the method will give reliable estimates of log K_{ow} for very hydrophobic chemicals, i.e, log $K_{ow} \geq 5$; and 3) the method is easier to carry out and takes less time to perform than the shake flask method.

In summary, the importance of K_{ow} in assessing the fate of substances released into the environment and the convenient trouble-free rapid procedures associated with the HPLC method make it ideally suited for use as a test method for neutral organic substances, and especially for very hydrophobic substances or multicomponent mixtures.

II. SCIENTIFIC ASPECTS

A. Rationale for the Test Method

The technique employed in this test method is referred to as reverse phase high pressure liquid chromatography (RP-HPLC). It involves the use of a chromatographic column that consists of an inert column support material onto which a non-polar C_{18} hydrocarbon is permanently bonded. Chemicals are injected onto this column and eluted with a polar solvent (in this case methanol-water). As the chemicals move along the column, they partition between the mobile phase (methanol-water) and the stationary hydrocarbon phase. Those chemicals that are more soluble in the hydrocarbon phase, that is more hydrophobic, will exhibit longer retention times (t_R) or be eluted more slowly than those that are more soluble in the polar methanol-water phase. Thus, the retention time of a chemical on the column is directly proportional to its hydrocarbon/water partition coefficient. This retention time can be correlated with the octanol/water partition coefficient when a calibration curve is constructed from the retention times of several reference chemicals for which K_{ow} has been established by using the conventional shake-flask method. The equation of the calibration curve has the following form:

$$\log K_{ow} = m \log t_R + b$$

McCall (1975) and Carlson et al. (1975) were the first to recognize the value of RP-HPLC for estimating K_{ow}. The technique was later verified by Mirrless et al. (1978), Unger et al. (1978), and Veith et al. (1979) whose work serves as the basis for the method described in this guideline.

B. Other Methods for Determining K_{ow}

1. The Conventional Shake Flask Method

The conventional method for determining the octanol/water coefficient involves shaking a solute with two immiscible solvents and then measuring the solute concentration in the two solvents after equilibration (Leo, et al. 1971). The distribution of the solute between the two solvents is a direct consequence of the thermodynamic requirements for equilibrium that apply only to dilute solutions. The resulting ratio of the two solute concentrations is the partition coefficient which is constant at a given temperature. Numerous researchers have used this method and published their results (Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971; Chiou et al. 1977). Both EPA (1982) and OECD (1981) specify the use of this method in their test protocols (see CG-1400). And, although there is no ASTM Standard Test Method for K_{ow}, the shake-flask method is the "standard method" used in industry when K_{ow} is to be determined.

As noted earlier, the shake flask method is not without problems, the most important of which are the formation of emulsions, surface adsorption of the solute, and the time consuming nature of the test procedure. However, with suitable care and patience these problems are minimized and basic experimental data is obtained that can be correlated with other important environmental fate parameters.

2. Estimation from Water Solubility

By definition, the partition coefficient expresses the equilibrium ratio of an organic chemical partitioned between an organic liquid (i.e., n-octanol) and water. This partitioning is, in essence, equivalent to partitioning of the organic chemical between itself and water. Thus, one would expect that a correlation might exist between the partition coefficient (K_{ow}) and water solubility(s). Indeed, as shown by Mackay (1977), both of these properties are a function of the aqueous phase activity coefficient of the compound, and the correlation between the K_{ow} and S is based partly on the relationship between this activity coefficient and its reciprocal.

Chiou et al. (1977) studied the relationship between K_{ow} and the water solubility, S, and found that, for 34 organic chemicals, an excellent linear correlation was observed between log K_{ow} and log S that extended to more than eight orders of magnitude in water solubility (10^{-3} to 10^{4} ppm), and six orders of magnitude in K_{ow} (10 to 10^7). From their data the following regression equation was derived:

$$\log K_{ow} = 5.00 - 0.670 \log S, \; r^2 = 0.970$$

where S is expressed μmol/L, and r^2 is the coefficient of determination.

To date a total of 18 different regression equations have been derived that correlate water solubility with the octanol water partition coefficient. This large number of equations results from the fact that solubility varies with the functional group of the molecule. Lyman et al. (1982) have summarized these equations along with the class and number of chemicals that apply to each equation, the respective correlation coefficient, the applicable range of S and K_{ow} values and the temperature at which the solubility data were obtained. Recent developments in the correlation between S and K_{ow} indicate that many of the above equations can be combined into a single general relationship (Lyman, 1984). This would greatly simplify the estimation procedure, but may introduce greater error, particularly for certain classes of compounds.

This method has a definite advantage in that K_{ow} can be estimated from an experimentally determined parameter - water solubility - whose test procedure involves less experimental difficulties. Although this approach also has a cost advantage in that two parameters are determined for the cost of one, it must be kept in mind that the K_{ow} is an estimated value subject to the limitations usually associated with approximations. Thus, while the estimation of K_{ow} from water solubility is definitely a valid method, the values obtained cannot be construed as being equivalent to those obtained from the conventional shake-flask method.

3. Estimation Using the Fragment Constant Method

Hansch and Leo (1979) have developed a method to estimate K_{ow} from empirically derived atomic or group fragment constants (f) and structural factors (F). Using these, the log K_{ow} is calculated using the following equation:

$$\log K_{ow} = \text{Sum of fragments (f)} + \text{structural factors (F)}$$

Of course, the critical piece of information required to apply this method is the structure of the chemical in question--which is not always known. However, if the structure is known then there are a total of over 200 fragments (f and F) available that take into account such structural factors as molecular flexibility, unsaturation, multiple halogenation, branching, and H-polar fragment interactions. These and the method of calculation, with examples, are reviewed extensively by Lyman et al. (1982). In addition, a large collection (about 15,000) of both measured and calculated K_{ow} values has been compiled by Hansch and Leo (1979); the method is also available for use on computer (Chou and Jurs 1979).

Estimation by the fragment constant is probably the best initial step in determining K_{ow} since it can be done without experimentation and is quite reliable for a large number of common organic chemicals. However, for some functional groups and complex or highly substituted molecules the fragment constant method gives erroneous, misleading results.

4. Estimation Using Thin-Layer Chromatography

It has been reported that thin-layer chromatography can be used to estimate K_{ow} (Mirrless et al. 1976; Hulshoff and Perrin 1976). However, high-pressure liquid chromatography (HPLC) is far superior to thin-layer chromatography (TLC) because of its accuracy (i.e., definition of the peak, reproducibility, ease of detection in many cases, and above all the range of applicability (HPLC is applicable over 5 orders of magnitude of K_{ow} while TLC is only applicable over 1.5 orders of magnitude of K_{ow}) (Mirrless et al. 1976).

C. Rationale for the Test Conditions

1. Chromatographic Column

Veith and Morris (1978) describe the use of either an analytical or preparative scale reverse phase column containing a solid support to which octadecylsilane has been permanently bonded. Both of these types columns are commercially available. The preparative scale column has been recommended for use with this test guideline because the authors found that this column produced linear calibration curves with shorter retention times using lower pressure than the analytical column.

2. Analytical Detector

Any suitable detector compatible with the HPLC and capable of detecting the test substance(s) can be used. This includes a uv detector which is sensitive to the detection of aromatic compounds; gas chromatography or liquid scintillation may be used for chemicals that do not absorb in the uv.

3. Purity of Solvents and Reagents

To prevent any interference with the determination of the retention time of the test substances, all solvents and reagents must be chromatographic or reagent grade. This includes the use of reagent grade water as described in ASTM 01193-77: "Standard Specification for Reagent Water."

4. Calibration Mixture

This test method requires that a calibration or standard curve be developed that relates the log K_{ow} to log t_R for a group of reference neutral organic chemicals. The K_{ow} for these chemicals must be initially determined using the standard shake flask method (CG-1400). The K_{ow} values should be well established (i.e., have small standard deviation) and cover the range from about 2 to 6 log units. This range can be extended and/or limited with the use of other standard substances. [Note: the calibration curve should have a high coefficient of determination, e.g., not less than r^2=0.975]. The standard substances recommended for use in this test guideline meet these criteria. The substances are dissolved in a suitable solvent and chromatographed prior to the test substances(s). Because of variations in the column, temperature, flow rate and solvent changes, the calibration curve must be determined daily.

D. Test Data and Reporting

1. Test Report

The data required in the test report are necessary to estimate K_{ow}, and evaluate the accuracy and precision of each step in the test procedure. These data include the following: the retention time for each of the substances in the calibration mixture, the K_{ow} and its logarithm, including the standard deviation, of each of the calibration substances as measured by the standard shake flask method, the regression equation (and coefficient of determination or correlation coefficient) that relates the logarithm of t_R and K_{ow}, the t_R and log t_R values along with the calculated log K_{ow} for each of the test substances. The following information is also required regarding the chromatograph: flow rate pressure and pH of eluent and the temperature at which the test was conducted.

2. Analytical Procedures

In order to assess the accuracy and precision of the HPLC employed, its detector and column, a detailed description of, or reference for these items must be provided. If any changes are made or problems encountered in the test method, these should be reported so that any necessary revisions can be incorporated in future editions.

III. REFERENCES

Carlson RM, Carlson RE, Kopperman HL. 1975. Determination of partition coefficients by liquid chromatography. J Chromatogr 107:219.

Chou JT, Jurs PC. 1979. Computer assisted computation of partition coefficients from molecular structure using fragment constants. J Chem Inf Comput Sci 19:172.

Chiou CT, Freed VH, Schmedding DW, Kohnert RL. 1977. Partition coefficient and bioaccumulation of selected organic chemicals. Environ Sci Technol 11:475.

EPA. 1982. Partition coefficient (n-octanol/water) CG-1400. Test Guideline. USEPA. Office of Toxic Substances. Washington DC.

Fujita T, Iwasa J, Hansch C. 1964. A new substituent constant, derived from partition coefficients. J Am Chem Soc 86:5175.

Hansch C, Anderson SM. 1967. The effect of intramolecular hydrophobic bonding on partition coefficients. J Org Chem 23:2583.

Hansch C, Leo A. 1979. Substituent constants for correlation analysis in chemistry and biology. New York: J. Wiley & Sons.

Hulshoff A, Perrin JH. 1976. A comparison of the determination of partition coefficients of 1,4-benzodiazepines by high-performance liquid chromatography and thin-layer chromatography. J Chromatogr 129:263.

Leo A, Hansch C, Elkins D. 1971. Partition coefficients and their uses. Chem Rev 71:525.

Lyman WJ. 1984. Personal communication.

Lyman WJ, Reehl WF, Rosenblatt DH. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw Hill Book Company. New York.

Mackay D. 1977. Environ Sci Technol 11:1219.

McCall JM. 1975. Liquid-liquid partition coefficients by high-pressure liquid chromatography. J Med Chem 18:549.

Mirrless MS, Moulton SJ, Murphy CT, Taylor PJ. 1976. Direct measurement of octanol-water partition coefficients by high-pressure liquid chromatography. J Med Chem 19:615.

OECD. 1981. Organization for Economic Cooperation and Development (OECD). Guidelines for Testing Chemicals: No. 107-Partition Coefficient (n-Octanol/Water). Director of Information, OECD; 2 Rue Andre-Pascal, 75775 PARIS CEDEX 16, France.

Twitchett PJ, Moffat AC. 1975. High pressure liquid chromatography of drugs: An evaluation of an octadecylsilane stationary phase. J Chromatogr 111:149.

Unger SH, Cook JR, Hollenberg JS. 1978. Simple procedures for determining octanol-aqueous partition, distribution, and ionization coefficients by reverse-phase high pressure liquid chromatography. J Pharm Sci 67 1364

Veith GD, Austin NM, Morris RT. 1979. A rapid method for estimating log P for organic chemicals. Water Res 13:43.

CG-6000
October, 1984

PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

DISCLAIMER

Certain commercial equipment, instruments, and materials are identified in this Test Guideline in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the Environmental Protection Agency, nor does it imply that the material, instruments, or equipment identified are necessarily the best available for the purpose.

28

Contents

Page

I. INTRODUCTION.. 1

A. Background and Purpose.......................... 1
B. Definitions and Units........................... 2
C. Principle of the Test Method.................... 4
D. Applicability and Specificity................... 9

II. TEST PROCEDURES....................................... 12

A. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-life in Sunlight................ 12

B. Tier 2 Test: Aqueous Photolysis in Sunlight....... 14

1. Test Conditions.............................. 14

a. Special Laboratory Equipment............... 14
b. Purity of Water............................ 14
c. Sterilization.............................. 15
d. pH Effects................................. 15
e. Volatile Chemical Substances............... 15
f. Control Solution........................... 16
g. Absorption Spectrum as a Criterion for Performing the Aqueous Photolysis Test... 17
h. Sunlight Actinometer....................... 17
i. Solar Irradiance Data...................... 19
j. Geometry of the Reaction Vessel............ 19
k. Chemical Analysis of Solutions............. 20

2. Preparations................................. 21

a. Preparation of Test Chemical Solution...... 21
b. Preparation of Buffer Solutions............ 22
c. Preparation of Actinometer Solution........ 22

3. Performance of the Tests..................... 23

a. Phase 1 Experiments........................ 23

i. Procedure 1............................. 24
ii. Procedure 2............................. 25
iii. Procedure 3............................. 25
iv. Analytical Methodology.................. 26

b. Phase 2 Experiments........................ 26

Page

III. DATA AND REPORTING.. 27

A. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-life in Sunlight................ 27

1. Treatment of Results........................... 27
2. Test Data Report............................... 28

B. Tier 2 Test: Aqueous Photolysis in Sunlight....... 30

1. Phase 1 Experiments............................ 30

a. Treatment of Results....................... 30
b. Specific Analytical and Recovery Procedures.............................. 32
c. Other Test Conditions...................... 33
d. Test Data Report........................... 33

2. Phase 2 Experiments............................ 34

a. Treatment of Results....................... 34
b. Other Test Conditions...................... 37
c. Test Data Report........................... 38

IV. REFERENCES... 40

V. APPENDICIES.. 41

A. Appendix A. Tables 1-6........................... 41
B. Appendix B. Illustrative Examples................ 52

1. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-life in Sunlight...................... 52

a. Illustrative Example 1..................... 52
b. Illustrative Example 2..................... 52

2. Tier 2, Phase 1: Aqueous Photolysis in Sunlight................................. 57

a. Illustrative Example 3..................... 57

3. Tier 2, Phase 2: Aqueous Photolysis in Sunlight................................. 60

a. Illustrative Example 4..................... 60

PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

I. INTRODUCTION

A. Background and Purpose

Numerous chemicals enter natural aquatic systems from a variety of sources. For example, chemical wastes are discharged directly into natural water bodies, and chemicals leach into natural water bodies from landfills. Pesticides are applied directly into water bodies, and are applied to soils and vegetation and subsequently leach into water bodies. Pollutants present in aqueous media can undergo photochemical transformation in the environment (i.e., in sunlight by direct photolysis or by sensitized photolysis). As a result, there is considerable interest in photolysis in solution, especially the photolysis of pesticides. However, most of these studies have been qualitative in nature and involved the identification of photolysis products. Quantitative data in the form of rate constants and half-lives are needed to determine the importance of photochemical transformation of pollutants in aqueous media. This Test Guideline describes a two-tiered screening level approach for determining direct photolysis rate constants and half-lives of chemicals in water in sunlight.

B. Definitions and Units

(1) "Radiant energy", or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.

(2) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_o) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ. Thus, $A_\lambda = \log_{10} (I_o/I)$.

(3) The "Beer-Lambert law" states that the absorbance of a solution of a given chemical species, at a fixed wavelength, is proportional to the thickness of the solution (ℓ), or the light pathlength, and the concentration of the absorbing species (C).

(4) "Molar absorptivity (ε_λ)" is defined as the proportionality constant in the Beer-Lambert law when the concentration is given in terms of moles per liter (i.e., molar concentration). Thus, $A_\lambda = \varepsilon_\lambda C\ell$, where A_λ and ε_λ represent the absorbance and molar absorptivity at wavelength λ and ℓ and C are defined in (3). The units of ε_λ are $\text{molar}^{-1}\ \text{cm}^{-1}$. Numerical values of molar absorptivity depend upon the nature of the absorbing species.

(5) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.

(6) The "half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical being tested to be reduced to one-half its initial value.

(7) The "sunlight direct aqueous photolysis rate constant (k_{pE})" is the first-order rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical dissolved in a water body in sunlight.

(8) The "solar irradiance in water (L_λ)" is related to the sunlight intensity in water and is proportional to the average light flux (in the units of 10^{-3} einsteins cm^{-2} day^{-1}) that is available to cause photoreaction in a wavelength interval centered at λ over a 24-hour day at a specific latitude and season date.

(9) "The Grotthus-Draper law", the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.

(10) The "Stark-Einstein law", the second law of photochemistry, states that only one molecule is

activated to an excited state per photon or quantum of light absorbed.

(11) The "reaction quantum yield (ϕ_λ)" for an excited state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ. It is the ratio of the number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ.

(12) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

As a convenient reference, a glossary of the important symbols used in this Test Guideline is given in Section IV of the Support Document [CS-6000].

C. Principle of the Test Method

This Test Guideline is based on the principles developed by Zepp and Cline (1977), Zepp (1978), the U.S. Environmental Protection Agency, Office of Toxic Substances [USEPA (1979)], Mill et al. (1981, 1982a, 1982b), and Dulin and Mill (1982).

Zepp and Cline (1977) published a paper on the rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water body. It has been shown that for the photolysis of a chemical in an optically thin aqueous solution, the kinetics of direct photolysis can be described by the following equations:

$$\ln(C_o/C_t) = k_{pE}t \tag{1}$$

$$t_{1/2E} = 0.693/k_{pE} \tag{2}$$

$$k_{pE} = \phi_E k_a \quad , \tag{3}$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, $k_a = \sum k_{a\lambda}$, the sum of $k_{a\lambda}$ values of all wavelengths of sunlight that are absorbed by the chemical (i.e., the light absorption rate constant), t is the time, C_o and C_t are the concentrations of chemical at t = 0 and t, and $t_{1/2E}$ represents the half-life. The term k_{pE} represents the first-order photolysis rate constant for a water body in sunlight in the units of reciprocal time.

Furthermore, under the same conditions cited above, the first-order direct photolysis rate constant, k_{pE}, is given by the equation

$$k_{pE} = \phi_E \sum \varepsilon_\lambda L_\lambda \quad , \tag{4}$$

where ϕ_E is the reaction quantum yield, ε_λ is the molar absorptivity in the units molar^{-1} cm^{-1}, L_λ is the solar irradiance in water in the units of 10^{-3} einsteins cm^{-2} day^{-1} [Mill et al. (1982a)], and the summation is taken over the range λ = 290 to 800 nm. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as natural water body. However, the method developed in this guideline measures rate constants in tubes (e.g., 13 x 100 mm) and the rate is faster in tubes. This is discussed in more detail in Section II.B.j. Thus, equations 1 and 2 have to be modified to take this into account. For simplicity, the following nomenclature is used. For water bodies, the rate constant is designated as k_{pE} with the subscript E designating rates in the environment in water bodies. For tubes, the rate constant is designated as k_p. The corresponding half-lives for water bodies and tubes are $t_{1/2E}$ and $t_{1/2}$, respectively. Thus, for tubes, equations 1 and 2 can be written as

$$\ln(C_o/C_t) = k_p t \quad ; \tag{5}$$

$$t_{1/2} = \frac{0.693}{k_p} \quad . \tag{6}$$

A simple first-tier screening test has been developed using equation 4. As an approximation, it is assumed that the reaction quantum yield ϕ_E is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 4 becomes

$$(k_{pE})_{max.} = \sum \varepsilon_\lambda L_\lambda \quad . \tag{7}$$

Using equation 7 in equation 2, the lower limit for the half-life is then given by

$$(t_{1/2E})_{min.} = \frac{0.693}{(k_{pE})_{max.}} \quad . \tag{8}$$

The molar absorptivity can be determined experimentally by the method outlined in Section II.A and the values of L_λ are given in Tables 3 to 6 as a function of latitude and season of the year. These data can then be used in equation 7 to calculate $(k_{pE})_{max.}$. Finally, $(k_{pE})_{max.}$ can then be substituted in equation 8 to calculate $(t_{1/2E})_{min.}$.

In a second-tier test method, an aqueous photolysis screening test has been developed to determine rate constants and half-lives in the presence of sunlight using equations 1, 2, 4, 5, and 6 [USEPA (1979), Mill et al. (1981, 1982a, 1982b), and Dulin and Mill (1982)]. The second-tier test method is divided into two phases. In phase one, the test chemical is photolyzed in sunlight in order to obtain an approximate rate constant, k_p^c. This method only gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis.

In phase two, a standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) is used to measure sunlight intensities incident on the sample during photolysis [Mill et al. (1982b) and Dulin and Mill (1982)]. The rate constant for this actinometer, k_p^a, can be adjusted to match the approximate rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the reaction quantum yield of the actinometer, the rate constant can be adjusted according to the equation

$$\phi_E^a = 0.0169[\mathrm{PYR}] \quad , \tag{9}$$

where [PYR] is the molar concentration of pyridine for a p-nitroacetophenone (PNAP) concentration of 1.00 x 10^{-5} M. The reaction quantum yield for the test chemical, ϕ_E^c, is given by

$$\phi_E^c = \frac{k_p^c}{k_p^a} \frac{\sum \epsilon_\lambda^a L_\lambda}{\sum \epsilon_\lambda^c L_\lambda} \phi_E^a \quad . \tag{10}$$

The reaction quantum yield of the test chemical, ϕ_E^c, can be determined in the following way. By measuring the concentration of test chemical and actinometer (PNAP) as a function of time t in sunlight, the ratio of rate constants, (k_p^c/k_p^a), can be determined using equation 5 (Section II.B.1.h). ϕ_E^a can be determined from equation 9 at the molar concentration of pyridine used in the standard actinometer. The term $\sum\varepsilon_\lambda^a L_\lambda$ for the actinometer has been tabulated as a function of latitude and season of the year [Table 2]. The term $\sum\varepsilon_\lambda^c L_\lambda$ for the test chemical can be obtained from the experimentally measured molar absorptivities (Section II.A) and the values of L_λ listed in Tables 3 to 6, as a function of latitude and season of the year.

With the values of ϕ_E^c, ε_λ^c, and the appropriate L_λ values, k_{pE} for the test chemical can be calculated as a function of latitude and season of the year in the United States using equation 4. The corresponding half-life can be calculated using k_{pE} in equation 2.

D. Applicability and Specificity

This Test Guideline is applicable to all chemicals which have uv-visible absorption maxima in the range 290-800 nm. Some chemicals have absorption maxima significantly below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, saturated alcohols, and saturated acids). This is a direct consequence of the Grotthus-Draper law of photochemistry. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals.

These test methods are only applicable to pure chemicals and not to the technical grade.

The first-tier screening test can be employed to estimate $(k_{pE})_{max.}$ and $(t_{1/2E})_{min.}$. If these data indicate that aqueous photolysis is an important process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.), then it is recommended that the second-tier photolysis tests be carried out to determine environmentally relevant rate constants and half-lives in sunlight. The data obtained from this test can be used to determine k_{pE} for the test chemical as a function of latitude and season of the year anywhere in the United States. These rate constants are in a form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

The second-tier screening test is applicable to the direct photolysis of chemicals in a homogeneous dilute solution with absorbance less than 0.05 in the reaction cell at all wavelengths greater than 290 nm and at shallow depths (less than 0.5 m). These results are applicable to direct sunlight photolysis for water bodies and clear sky conditions. In addition, these experiments are limited to the direct photolysis of chemicals in air-saturated pure water.

This screening test has been designed to determine the molar absorptivity of a test chemical, $\varepsilon_{\lambda}^{C}$, and its reaction quantum yield, ϕ_{E}^{C}. These parameters can be used to determine environmentally relevant rate constants at low absorbance and shallow depths in pure water as a function of latitude and season of the year. Tables of solar irradiance (Table 3-6) have been included in this Test Guideline to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp and Cline (1977) have written a computer program to calculate the rates of photolysis as a function of depth in water, as a function of the attenuation coefficient of the water (α_{λ}) for natural water bodies, the average ozone layer thickness that pertains to the seasons and location of interest, and as a function of latitude and season of the year. This program has been recently updated with the best available solar irradiance data and is called the GC SOLAR program. The GC SOLAR computer program is available on request. [R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.]

II. TEST PROCEDURES

A. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-Life in Sunlight

The uv-visible absorption spectra in aqueous solution can be determined by the methods described in Test Guideline CG-1050. It is recommended that the following additional procedures be followed:

(1) For chemicals which ionize or protonate (e.g., carboxylic acids, phenols, amines), carry out uv-visible absorption studies at pHs at least two orders or magnitude above the pK_a and at least two orders of magnitude below the pK_a. Prepare buffer solutions at 25°C using reagent grade chemicals and distilled water as follows:

pHs in the range 3-6*: NaH_2PO_4
HCl

pHs in the range 6-8* KH_2PO_4
$NaOH$

---

*Use the minimum concentration of buffers to attain the desired pH.

pHs in the range >8: Prepare buffers as described in the Handbook of Chemistry and Physics.

Check the pH of all the buffer solutions with a pH meter at 25°C and adjust to the proper pH, if necessary. These buffer solutions can then be added to the test chemical solution until the desired pH is obtained. If these buffers are inadequate, then adjust the pH of the test chemical solution with 1 M HCl or NaOH at 25°C.

(2) Measure the absorbance, A_λ, as a function of wavelength in the range 290-800 nm in duplicate. If applicable, measure A_λ at each experimental pH. Record, in duplicate, the baseline when both the sample and reference cells are filled with blank solutions. These data will be used to calculate the molar absorptivities for the appropriate wavelength intervals and wavelength centers, Table 1 (Section V.A.), where the test chemical absorbs light. The wavelength center is defined as the midpoint of the interval range.

It must be emphasized that the molar absorptivities of the test chemical must be carefully determined, especially in the tails of the absorption bands at $\lambda > 290$ nm. Large errors will be encountered in calculating photolysis rate constants and half-lives if these measurements are not carefully carried out.

B. Tier 2 Test: Aqueous Photolysis in Sunlight

1. Test Conditions

a. Special Laboratory Equipment

It is recommended that quartz tubes be used for the photolysis of chemicals with appreciable absorption at wavelengths below 340 nm. Chemicals that absorb appreciably at wavelengths greater than 340 nm may be tested in borosilicate tubes. Thin-walled borosilicate or quartz tubes are recommended. Disposable culture tubes (13 x 100 mm) with Teflon-lined screw caps or quartz tubes with quartz or borosilicate stoppers, Teflon-lined, may be used as reaction vessels. Tubes of 11 mm i.d. are recommended. For some chemicals, it may be difficult to determine the concentration of the test chemical in reaction tubes of small volume. For these chemicals, larger volume reaction vessels are recommended provided that the cell walls are thin and the pathlength of radiation through the vessel is less than 0.5 meter.

b. Purity of Water

Reagent grade water, e.g., water meeting ASTM Type II A standards, or an equivalent grade, is highly recommended to minimize biodegradation. ASTM Type II A water is described in ASTM D 1193-77, "Standard Specification for Reagent Water". Air-saturated water can be easily prepared by allowing the water to equilibrate in a vessel plugged with sterile cotton.

c. Sterilization

It is extremely important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions and in carrying out all photolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable non-chemical method.

d. pH Effects

It is recommended that all photolysis experiments be carried out at pHs at least two orders of magnitude above the pK_a and at least two orders of magnitude below the pK_a for any chemical which ionizes or protonates (e.g., carboxylic acids, phenols, and amines). Buffers described in Section II.B.2.b. should be used.

e. Volatile Chemical Substances

Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels.

Disposable culture tubes with Teflon-lined screw caps or quartz tubes with quartz or borosilicate stoppers, Teflon-lined, are recommended. Volatile compounds can be conveniently studied in culture tubes equipped with Mininert® valves. Samples can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate. As an alternative, the tubes can be sealed with a torch. In addition, the reaction vessels should be as completely filled as is possible to prevent volatilization to any air space.

f. Control Solution

It is extremely important to take certain precautions to prevent loss of chemical from the reaction vessels by processes other than photolysis. For example, biodegradation and volatilization can be eliminated or minimized by use of sterile conditions and minimal air space in sealed vessels. Hydrolysis is a process which cannot be minimized by such techniques. Thus, control vessels containing test substance which are not exposed to sunlight are required. In this way, the loss of test chemical for processes other than photolysis may be determined and eliminated. For simplicity, if the loss of chemical in the control is small (i.e., approximately 10% or less), one can calculate a first-order loss, k_{loss}, and subtract it from $(k_p)_{obs.}$ to give the corrected direct photolysis rate constant k_p. If hydrolysis is found to be significant (i.e., greater than

46

10%), hydrolysis studies should be carried out first. [Test Guideline CG-5000].

g. Absorption Spectrum as a Criterion for Performing the Aqueous Photolysis Test

This aqueous photolysis screening test is applicable to all chemicals which have uv-visible absorption maxima in the range 290-800 nm. Some chemicals have absorption maxima significantly below 290 nm but have measureable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals. The absorption spectrum of the chemical in aqueous solution can be measured by Test Guideline CG-1050.

h. Sunlight Actinometer

In order to quantify the rate of photolysis more precisely, it is necessary to measure the sunlight intensity incident on the sample during photolysis. A standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) has been developed [Mill et al. (1981, 1982b); Dulin and Mill (1982)] to measure the sunlight intensity incident on the sample during photolysis and this actinometer has been incorporated in this Test Guideline. According to equation 4, the rate constant is a function of the reaction quantum yield. Furthermore, the reaction quantum yield can be adjusted by varying the molar concentration of the pyridine according to equation 9. Hence, by varying the pyridine concentration, the actinometer photolysis rate constant can be adjusted so that the half-life can range from several hours to several weeks. The initial concentration of PNAP is set at 1.00 x 10^{-5} M.

Using the test chemical photolysis rate constant, k_p^c, determined in Tier 2, Phase 1, and the variable $k_a^a (= \sum \varepsilon_\lambda^a L_\lambda)$, listed in Table 2, the molar concentration needed to adjust the rate of disappearance of PNAP in PNAP/PYR to match the rate of disappearance of the test chemical is given by

$$[\mathrm{PYR}] = 26.9\ (k_p^c/k_a^a) \quad . \tag{11}$$

Experiments are caried out by simultaneously photolyzing the test chemical and actinometer solutions. The concentrations of test chemical and actinometer are measured periodically as a function of time. These data are then used to determine the ratio of the rate constants, k_p^c/k_p^a, using linear regression analysis on the following equation

$$\ln(C_o/C_t)^c = (k_p^c/k_p^a)\ \ln(C_o/C_t)^a \quad , \tag{12}$$

with $\ln\ (C_o/C_t)^a$ as the independent variable and $\ln(C_o/C_t)^c$ as the dependent variable. The slope of the best straight line is the ratio of the rate constants, k_p^c/k_p^a.

i. Solar Irradiance Data

In order to calculate the reaction quantum yield of the test chemical, ϕ^{c}_{E}, and then calculate k^{c}_{pE} and $t_{1/2E}$, it is $t_{1/2E}$ necessary to use the solar irradiance parameter L_λ. L_λ values are proportional to the average light flux that is available to cause photolysis in a wavelength interval centered at λ over a 24-hour day at a specific latitude and season date. The L_λ values are defined by the angle of declination of the sun at -20° for winter, -10° for fall, +10° for spring and +20° for summer. The actual dates for 1982 that correspond to these angles of declination are January 21, April 16, July 24, and October 20, for winter, spring, summer and fall, respectively [AA (1982)]. The L_λ values for these season dates are listed in Tables 3 to 6 (Section V.A) as a function of latitude and are applicable to clear sky conditions, water bodies, shallow depths, and for chemicals whose absorbance is less than 0.05 in pure water [Mill et al. (1984)].

j. Geometry of the Reaction Vessel

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body while the method developed in this Test Guideline measures rate constants (k_p) in tubes (e.g., 13 x 100 mm). However, rates in tubes are faster than in water bodies and it has been experimentally observed [Mill et al. (1982b)] that

$$k_p = 2.2k_{pE} \quad . \tag{13}$$

Because tubes are the simplest and easiest reaction vessels to use, this Test Guideline recommends the use of tubes as reaction vessels and the method has been modified to take into account the increased rate in tubes (equation 13).

k. Chemical Analysis of Solutions

In determining the concentration of the chemical in solution, an analytical method should be selected which is most applicable to the analysis of the specific chemical substance. Chromatographic methods are generally recommended because of their chemical specificity in analyzing the parent chemical substance without interference from impurities. Whenever practicable the chosen analytical method should have a precision of ± 5 percent or better.

The p-nitroacetophenone in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is

2.5% acetic acid, 50% acetonitrile, and 47.5% water which is passed through the column at a flow rate of 2 mL/minute.

2. Preparations

a. Preparation of Test Chemical Solution

Prepare homogeneous solutions with the chemical at less than one-half of its solubility in water and at a concentration such that the absorbance is less than 0.05 in the photolysis reaction vesel at wavelengths greater than 290 nm. For very hydrophobic chemicals, it is difficult and time consuming to prepare aqueous solutions. To facilitate the preparation of aqueous solutions containing very hydrophobic chemicals and to allow for easier analytical measurement procedures, the following procedure may be used to aid in the dissolution of the chemical. Dissolve the pure chemical in reagent grade acetonitrile. Add pure water as described under Test Conditions, Section II.B.1.b, or buffer solution as described under Preparations, Section II.B.2.b., for chemical substances which ionize or protonate, to an aliquot of the acetonitrile solution. Do not exceed one volume-percent of acetonitrile in the final solution. Place the reaction solution in the appropriate photolysis reaction tubes as described in Section II.B.1.a.

b. Preparation of Buffer Solutions

Prepare buffer solutions according to the procedures outlined in Section II.A.1 using reagent grade chemicals and pure water as described under Test Conditions, Section II.B.1.b.

c. Preparation of Actinometer Solution

Using the test chemical photolysis rate constant, k_p^c, determined in Tier 2, Phase 1, and the variable k_a^a listed in Table 2, the molar concentration of pyridine needed to adjust the rate of disappearance of p-nitroacetophenone (PNAP) to match the rate of disappearance of the test chemical can be obtained from equation 11. The variable k_a^a (= $\sum \varepsilon_\lambda^a L_\lambda$) is equal to the day-average rate constant for sunlight absorption by PNAP which changes with season and latitude. The value of k_a^a is selected from Table 2 for the season nearest the mid-experiment date of the Tier 2, Phase 1, studies and the decadic latitude nearest the latitude of the experimental site.

Once the molar concentration of pyridine [PYR] has been determined, an actinometer solution can be prepared as follows. Dissolve 0.165 gms. of PNAP in 100 mL of acetonitrile (0.01 M). Add 1 mL of this solution to a one liter volumetric flask. Add to the volumetric flask the mass in grams, or the volume (V) of pyridine at 20°C, obtained from the equations

$$\text{mass(grams)} = 79.1[\text{PYR}]$$

$$V(\text{mL}) = 80.6[\text{PYR}] \quad . \tag{14}$$

Fill the volumetric flask with pure water (Section II.B.1.b.) to give one liter of solution and shake vigorously to make sure that the solution is homogeneous. The PNAP/PYR solution should be wrapped with aluminum foil and kept from bright light.

3. Performance of the Tests

a. Phase 1 Experiments

For all experiments, prepare an aqueous solution of the chemical substance, as described in Section II.B.2.a., and a sufficient number of samples in quartz or borosilicate glass tubes to perform all the required tests. Fill the tubes as completely as possible and seal them. Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminum foil or by other suitable methods. These samples are analyzed for the chemical substance immediately after completion of the experiment to measure the loss of chemical in the absence of light. Place the samples, including the controls, outdoors in an area free of shade and reflections of sunlight from windows and buildings. Place the samples on a black, non-reflective background and inclined at approximately 30° from the horizontal with the upper end pointing due north (in the northern hemisphere). Conduct the photolysis experiments during a frost-free time of year (e.g., May, June, July, August, or September in the northern hemisphere -- weather permitting) and start the experiments initially at noon (1200 hours). Record the date and time the experiment was begun, the date and time completed, the time of sunrise and sunset on all days when photolysis experiments were performed, the times exposure was stopped and restarted for intermittent exposure, the weather conditions during the period, and the latitude of the site. For chemical substances that ionize or protonate, carry out photolysis experiments at the required pHs as described under Test Conditions, Section II.B.1.d.

If a significant loss of test chemical has occurred in the control samples, determine the cause and eliminate or minimize the loss. If hydrolysis is found to be significant, hydrolysis studies should be carried out first (Section II.B.1.f.).

Use one of the following procedures, depending on how fast the chemical substance photolyzes.

i. Procedure 1

If the chemical substance transforms 50-80% within 28 days, measure the concentration of the chemical substance, in duplicate, at time t = 0 and periodically (at least four data points at approximately equal time intervals) at noon (1200 hours) until at least 50% of the substance has been consumed. As a simplification, the sampling times can be estimated as the photolysis experiments are in progress. Determine the concentration of test chemical from two, freshly opened, reaction tubes for each time point. Determine the concentration in each of the two control solutions as soon as the photolysis experiments are completed.

ii. Procedure 2

If the chemical substance transforms in the range of 20-50% in 28 days, determine the concentration of the chemical substance, in duplicate, at time t = 0. Determine the concentration of the chemical in the two separate reaction tubes and the two control tubes after 28 days of photolysis.

iii. Procedure 3

For chemical substances that transform in sunlight 50-80% within one or two days, place the samples outside at noon (1200 hours) and analyze two samples for the concentration of the chemical substance at t = 0, and in two, freshly opened, reaction tubes at noon (1200 hours) the next day, and again, in two, freshly opened, reaction tubes at noon (1200 hours) the second day. Determine the concentration of the test chemical in each of the two control solutions after the first day of photolysis and as soon as the photolysis experiments have been completed on the second day.

iv. Analytical Methodology

Select an analytical method which is most applicable to the analysis of the specific chemical being tested (Section II.B. 1. k.).

b. Phase 2 Experiments

Using the test chemical photolysis rate constant, k_p^c, determined in Tier 2, Phase 1, prepare an actinometer solution, as described in Section II.B.2.c., and a sufficient number of samples in quartz tubes to perform all the required tests. Fill all the tubes as completely as possible, seal them, and cover them with aluminum foil as soon as possible after preparation. Prepare an aqueous solution of test chemical, as described in Section II.B.2.a., and a sufficient number of samples in quartz or borosilicate tubes to perform all the required tests. Fill these tubes as completely as possible, seal them, and cover them with aluminum foil as soon as possible after preparation. Place all the samples outdoors in an area free of shade and reflections of sunlight from windows and buildings. Place the samples on a black, nonreflective background and inclined at approximately 30° from the horizontal with the upper end pointing due north (in the northern hemisphere). Remove the foil from all samples except for the test chemical control solutions and the actinometer control solutions at noon (1200 hours). Based on the results of the Phase 1 experiments, determine the concentration of test chemical and actinometer (PNAP), in triplicate, at time t = 0 and periodically (at least five data points at approximately equal time intervals). Determine the concentration of PNAP in the three actinometer control solutions and the concentration of test chemical in the three control solutions for each time point.

Select an analytical method which is most applicable to the analysis of the specific chemical tested, Section II.B.1.k., and follow the procedure given in Section II.B.1.k. for the analysis of PNAP.

III. DATA AND REPORTING

A. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-Life in Sunlight

1. Treatment of Results

The molar absorptivity can be determined from the absorption spectra using the expression

$$\varepsilon_{\lambda}^{C} = A_{\lambda}/C\ell \quad , \qquad (15)$$

where A_{λ} is the absorbance at wavelength λ, C is the molar concentration of test chemical, and ℓ is the cell pathlength in centimeters. The molar absorptivity of the chemical should be determined for the wavelengths listed in Table 1 for a solution of concentration C and in a cell with pathlength ℓ. If the absorption curve is flat within the interval around the wavelength λcenter, ε_λ may be determined from the absorbance A_λ at λcenter using equation 15. If a large change in absorbance occurs within this interval, obtain an average absorbance A_λ at λcenter based on the absorbances at the two boundaries of the interval. Calculate an average ε_λ using the average value of A_λ in equation 15. Determine the molar absorptivity for each replicate and calculate a mean value.

Using the molar absorptivities obtained from the spectra and the values of the L_λ from Tables 3 to 6, the maximum rate constant $(k_{pE})_{max.}$ can be calculated at a specific latitude and season of the year using equation 7. The minimum half-life, $(t_{1/2E})_{min.}$ can then be calculated using this $(k_{pE})_{max.}$ in equation 8.

Two hypothetical examples are presented in an Appendix, Section V.B.1, to illustrate how the test data obtained in the first-tier screening test can be used.

2. Test Data Report

(1) Submit the original chart, or photocopy, containing a plot of absorbance of test chemical vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10 nm

intervals. Each spectrum should be clearly marked with the test conditions.

(2) Report the concentration of the test chemical solution, the type of absorption cell used (quartz or borosilicate glass) and the pathlength.

(3) Report A_λ and ε_λ at λcenter for each replicate and the mean value.

(4) Report $(k_{pE})_{max.}$ and $(t_{1/2E})_{min.}$ for the summer and winter solstices using the appropriate L_λ values from Tables 3-6 closest to the latitude of the chemical manufacturing site.

(5) Report the identity and composition of the solvent used in the spectral absorption study.

(6) For ionizable chemicals, report its pK_a. Report the type and concentration of the buffers employed for each pH. Report the pHs in which the photolysis experiments were carried out.

(7) Describe the method employed in determining the test chemical's concentration.

(8) Report the name, structure, and purity of the test chemical.

(9) Submit a recent test spectrum on appropriate reference chemicals for photometric and wavelength accuracy.

(10) Report the name and model of the spectrophotometer used.

(11) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, gain, etc.

B. Tier 2 Test: Aqueous Photolysis in Sunlight

1. Phase 1 Experiments

a. Treatment of Results

If a small loss of test substance in the control tubes has occurred, use this data to make corrections to the measured photolysis rate (Section II.B.1.f.). Note the site of photolysis and its latitude and the weather conditions. For procedures 1 and 2 note the dates and times of actual exposure including times of sunrise and sunset and, in case the cells are moved to prevent freezing or for other reasons, make sure that these times are recorded and that the cells are kept in a dark place when exposure is not in progress.

(1) For chemical substances which transform 50-80% within 28 days, use a concentration C_t, which corresponds to less than 50% of the initial concentration of chemical substance remaining, and the corresponding time t, in days, along with the initial molar concentration C_o, in equation 5 to calculate k_p in days^{-1}. From the

analysis of the two samples at time $t = 0$ and t, calculate a mean value of C_o and C_t, respectively, and a value of k_p. If a slight loss of chemical has been detected in the controls, then calculate a rate constant as follows: Calculate an average concentration C_t, based on the duplicate measurements of concentration in the controls. Use this concentration along with the average initial concentration in equation 5 and calculate a rate constant k_{loss}. Using this rate constant along with the observed rate constant, the corrected rate constant is then

$$k_p = (k_p)_{obs.} - k_{loss} \quad . \tag{16}$$

Calculate the half-life, $t_{1/2}$ using the corrected k_p value in equation 6;

(2) for chemical substances which transform 20-50% in 28 days, use the mean concentration C_t remaining at t = 28 days along with the mean value of C_o to calculate k_p. Use the same procedure as described above to calculate the value of k_p and $t_{1/2}$. If less than 20% of the chemical substance degrades in 28 days, report the mean concentration of C_t and C_o. In this case the apparent half-life is reported as greater than 3 months; and

(3) for chemical substances which transform 50% or more in the first day, as described in procedure 3, calculate a full day k_p value using the mean concentration C_t of chemical substance remaining at noon (1200 hours) after the first day along with the mean value of C_o using equation 5. For chemical substances which degrade less than 50% at noon (1200 hours) after the first day but 50% or more at noon (1200 hours) the second day, calculate k_p using the mean concentration of chemical substances remaining at noon (1200 hours) the second day. Calculate the half-life, $t_{1/2}$, using the mean value of k_p in Equation 6. If a small loss of test substance in the control tubes has occurred, use this data to make corrections to the mesured photolysis rate as described above. Note the dates of photolysis, the latitude, and the site.

A hypothetical example is presented in an Appendix, Section V.B.2, to illustrate how the test data obtained in the Tier 2, Phase 1, test method can be used.

b. Specific Analytical and Recovery Procedures

(1) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision; and

C2

(2) if extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

c. Other Test Conditions

(1) Report the size, approximate cell wall thickness, and type of glass used for the reaction tubes;

(2) report the initial pH of all test solutions, if appropriate;

(3) for all procedures, report the dates of photolysis, the time of sunrise and sunset on each photolysis day, the site of photolysis and its latitude, and the weather conditions. For procedures 1 and 2 submit the dates and times of actual exposure, and the duration of exposure, and, for intermittent exposure, the fraction of each day during which photolysis occurred;

(4) if acetonitrile was used to solubilize the test substance, report the percent, by volume; and

(5) if a significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

d. Test Data Report

(1) For each photolysis experiment, report:

i. The initial molar concentration of test chemical (C_o) of each replicate and the mean value.

ii. The molar concentration of test chemical for each replicate and the mean value for each time point t.

iii. The molar concentration of each replicate control sample and the mean value after completion of the photolysis experiments.

(2) For procedure 1, 2, or 3, report the value of k_p. If small losses of chemical are observed, report $(k_p)_{obs.}$, k_{loss} and k_p. Report the half-life ($t_{1/2}$) calculated using the value of k_p.

2. Phase 2 Experiments

a. Treatment of Results

The objectives of this set of experiments is to determine the sunlight reaction quantum yield, ϕ_E^c, for a specific test chemical. ϕ_E^c can be calculated using equation 10,

$$\phi_E^c = \frac{k_p^c}{k_p^a} \frac{\sum \varepsilon_\lambda^a L_\lambda}{\sum \varepsilon_\lambda^c L_\lambda} \phi_E^a \quad , \tag{10}$$

by the following steps:

(1) Determine the ratio of the rate constants, k_p^a/k_p^c, as described in Section II.B.1.h using equation 12. If a slight loss of test chemical or actinometer (PNAP) was detected in the controls at any time t, then employ the following procedure. Consider, as an example, the loss of test chemical in the control at time t. Using the average concentration of the test chemical in the controls from the replicates at time t and the average initial concentration, calculate $\ln(C_o/C_t)^c{}_{loss}$. Using the average concentration of test chemical from the replicates after photolysis time t, calculate $\ln(C_o/C_t)^c{}_{obs.}$. The corrected term is then

$$\ln(C_o/C_t)^c{}_{corr.} = \ln(C_o/C_t)^c{}_{obs.} - \ln(C_o/C_t)^c_{loss}. \quad (17)$$

The same procedure can be applied to obtain a corrected term from the actinometer (PNAP). Using the corrected terms for test chemical and/or actinometer in equation 12, determine the ratio of the rate constants (k_p^a/k_p^c) as described in Section II.B.1.h.

(2) Determine the quantum yield of the actinometer, ϕ_E^a, using equation 9 and the molar concentration of pyridine [PYR] present in the actinometer,

(3) Determine the value of $\sum\varepsilon_\lambda^c L_\lambda$ for the test chemical as follows: the molar absorptivities, ε_λ^c, have

been determined by the procedure given in Section II.A. and the results have been tabulated according to Section III.A.2. Choose the appropriate L_λ values (Tables 3 to 6) that correspond to the season closest to the season in which the Phase 2 experiments were performed and to the latitude nearest the latitude of the experimental site. Calculate the product of ε_λ^c and L_λ for each wavelength interval where ε_λ has a nonzero value. Sum the products of $\varepsilon_\lambda L_\lambda$ over all wavelength intervals.

(4) Determine the value of $\sum \varepsilon_\lambda^a L_\lambda$, for the actinometer, as follows: These values have been calculated and are given in Table 2. Choose the appropriate value that corresponds to the season closest to the season in which the Phase 2 experiments were performed and to the latitude nearest the latitude of the experimental site.

(5) Substitute the values of k_p^a/k_p^c, ϕ_E^a, $\sum \varepsilon_\lambda^a L_\lambda$, and $\sum \varepsilon_\lambda^c L_\lambda$ in equation 10 and calculate ϕ_E^c, the quantum yield of the test chemical in the environment (i.e., in sunlight).

Once ϕ_E^c has been determined, equation 4 can be used to calculate k_{pE} at any season of the year and latitude using the measured values of the molar absorptivities, ε_λ^c, and the appropriate L_λ values (Tables 3 to 6). The half-life can then be calculated using k_{pE} in equation 2.

A hypothetical example is presented in an Appendix, Section V.B.3, to illustrate how the test data obtained in the Tier 2, Phase 1, test method can be used.

b. Other Test Conditions

(1) Report the size, approximate cell wall thickness, and type of glass used for tubes to hold the test chemical and actinometer solutions.

(2) Report the initial pH of all test chemical solutions, if appropriate, and the type and concentration of the buffers employed for each pH.

(3) If acetonitrile was used to solubilize the test chemical, report the percent, by volume, of the acetonitrile, which was used.

(4) If significant loss of test chemical occured in the control solution, indicate the causes and how they were eliminated or minimized.

C7

c. Test Data Report

(1) Report the initial molar concentration of chemical (C_o) of each replicate and the mean value.

(2) Report the initial molar concentration of PNAP and the molar concentration of pyridine used in the actinometer.

(3) Report the time and date the sunlight photolysis experiments were started, the time and date the experiments were completed, and the elapsed photolysis time in days.

(4) For each time point, report the three separate values for the molar concentration of test chemical and PNAP and the mean values.

(5) For each time point, report the three separate values of the molar concentration of test chemical and PNAP for the controls and the mean values.

(6) Tabulate and report the following data: t, $\ln(C_o/C_t)^c$, and $\ln(C_o/C_t)^a$. From the linear regression analysis, report the ratio of the rate constants, k_p^c/k_p^a, and the correlation coefficient.

(7) If loss of test chemical and/or actinometer was observed during photolysis, then report the data $\ln(C_o/C_t)_{corr.}$, $\ln(C_o/C_t)_{obs.}$, $\ln(C_o/C_t)_{loss}$ for the test chemical and/or actinometer at each time t. From the linear regression analyis of $\ln(C_o/C_t)^c{}_{corr.}$ and

C8

$\ln(C_o/C_t)^a_{corr.}$, report the ratio of the rate constants, k^c_p/k^a_p and the correlation coefficient.

(8) Report the reaction quantum yield of the actinometer (ϕ^a_E).

(9) Report the value of k^a_a for the actinometer corresponding to the season closest to the season in which the photolysis experiments were carried out and to the latitude nearest the latitude of the experimental site.

(10) Tabulate the values of λcenter, ε^c_λ, L_λ, and $\varepsilon^c_\lambda L_\lambda$ for the test chemical corresponding to the season closest to the season in which the photolysis experiments were carried out and to the latitude nearest the latitude of the experimental site.

(11) Report the value $\sum\varepsilon^c_\lambda L_\lambda$ for the test chemical from step 10.

(12) Report the reaction quantum yield of the test chemical.

(13) Report k_{pE} and $t_{1/2E}$ for the summer and winter seasons using the appropriate L_λ values from Tables 3-6 closest to the latitude of the chemical manufacturing site.

(14) For chemicals that ionize, report the data for steps 1-13 for the experiments at the required pHs.

IV. REFERENCES

AA. 1982. Astronomical Almanac.

ASTM. 1978. Annual book of ASTM standards, American Society for Testing and Materials, Philadelphia, PA., Part 31, Method D 1193-77.

Dulin D and Mill T. 1982. Development and application of solar actinometers. Environ Sci and Technol 16:815.

Handbook of Chemistry and Physics. The Chemical Rubber Co. Cleveland, Ohio.

Mill T, Davenport SE, Dulin DE, Mabey WR, and Bawol R. 1981. Evaluation and optimization of photolysis screening protocols. EPA-560/5-81-003.

Mill T, Mabey WR, Bomberger DC, Chou T-W, Hendry DG, and Smith JH. 1982a. Laboratory protocols for evaluating the fate of organic chemicals in air and water. EPA-600/3-82-022.

Mill T, Mabey WR, Hendry DG, Winterle J, Davenport J, Barich V, Dulin D, and Tse D. 1982b. Design and validation of screening and detailed methods for environmental processes. EPA-

Mill T, Davenport JE, Winterle JS, Mabey WR, Drossman H, Tse D, and Liu A. 1984. Toxic substances process data generation and protocol development. EPA Contract No. 68-03-2981 with EPA Athens Research Laboratory, Office of Research and Development. Draft final report.

USEPA. 1979. U.S. Environmental Protection Agency. Office of Toxic Substances. Toxic Substances Control: Discussion of premanufacture testing policy and technical issues. Request for comment. Federal Register 44, 16240.

Zepp RG and Cline DM. 1977. Rates of direct photolysis in aquatic environment. Environ Sci and Technol 11:359.

Zepp RG. 1978. Quantum yields for reaction of pollutants in dilute aqueous solution. Environ Sci and Technol 12:327.

70

V. APPENDICES

A. Appendix A. Tables 1-6

TABLE 1. WAVELENGTH CENTER AND INTERVALS FOR L_λ

λcenter (nm)	Interval Range From (nm)	To (nm)	$\Delta\lambda$ (nm)
297.5	296.2	298.7	2.5
300.0	298.7	301.2	2.5
302.5	301.2	303.7	2.5
305.0	303.7	306.2	2.5
307.5	306.2	308.7	2.5
310.0	308.7	311.2	2.5
312.5	311.2	313.7	2.5
315.0	313.7	316.2	2.5
317.5	316.2	318.7	2.5
320.0	318.7	321.2	2.5
323.1	321.2	325.0	3.8
330.0	325.0	335.0	10.0
340.0	335.0	345.0	10.0
350	345.0	355.0	10.0
360	355.0	365.0	10.0
370	365.0	375.0	10.0
380	375.0	385.0	10.0
390	385.0	395.0	10.0
400	395.0	405.0	10.0

TABLE 1 (continued)

λcenter (nm)	Interval Range From (nm)	To (nm)	Δλ (nm)
410	405.0	415.0	10.0
420	415.0	425.0	10.0
430	425.0	435.0	10.0
440	435.0	445.0	10.0
450	445.0	455.0	10.0
460	455.0	465.0	10.0
470	465.0	475.0	10.0
480	475.0	485.0	10.0
490	485.0	495.0	10.0
500	495.0	505.0	10.0
525	512.5	537.5	25
550	537.5	562.5	25
575	562.5	587.5	25
600	587.5	612.5	25
625	612.5	637.5	25
650	637.5	662.5	25
675	662.5	687.5	25
700	687.5	712.5	25
750	725.0	775.0	50
800	775.0	825.0	50

TABLE 2. DAY AVERAGED RATE CONSTANT (k_a^a)* FOR SUNLIGHT ABSORPTION BY PNAP AS A FUNCTION OF SEASON AND DECADIC LATITUDE

	Season			
Latitude	Spring	Summer	Fall	Winter
20°N	515	551	409	327
30°N	483	551	333	233
40°N	431	532	245	139
50°N	362	496	154	64

---

*$k_a^a = \sum \varepsilon_\lambda^a L_\lambda$ in day^{-1}

TABLE 3. L_λ VALUES FOR LATITUDE 20°N[a,b,c]

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	1.10 (-4)	1.52 (-4)	7.77 (-5)	3.71 (-5)
300.0	4.06 (-4)	5.26 (-4)	2.96 (-4)	1.62 (-4)
302.5	1.10 (-3)	1.35 (-3)	8.21 (-4)	4.99 (-4)
305.0	2.37 (-3)	2.79 (-3)	1.79 (-3)	1.17 (-3)
307.5	4.24 (-3)	4.86 (-3)	3.24 (-3)	2.25 (-3)
310.0	6.65 (-3)	7.45 (-3)	5.13 (-3)	3.72 (-3)
312.5	9.42 (-3)	1.04 (-2)	7.33 (-3)	5.47 (-3)
315.0	1.24 (-2)	1.35 (-2)	9.68 (-3)	7.40 (-3)
317.5	1.54 (-2)	1.66 (-2)	1.21 (-2)	9.38 (-3)
320.0	1.82 (-2)	1.96 (-2)	1.44 (-2)	1.13 (-2)
323.1	3.23 (-2)	3.45 (-2)	2.55 (-2)	2.04 (-2)
330.0	1.10 (-1)	1.17 (-1)	8.75 (-2)	7.08 (-2)
340.0	1.37 (-1)	1.45 (-1)	1.10 (-1)	9.02 (-2)
350.0	1.52 (-1)	1.60 (-1)	1.22 (-1)	1.01 (-1)
360.0	1.67 (-1)	1.76 (-1)	1.35 (-1)	1.12 (-1)
370.0	1.78 (-1)	1.88 (-1)	1.45 (-1)	1.21 (-1)
380.0	1.89 (-1)	2.00 (-1)	1.55 (-1)	1.30 (-1)
390.0	1.79 (-1)	1.89 (-1)	1.46 (-1)	1.22 (-1)
400.0	2.57 (-1)	2.71 (-1)	2.09 (-1)	1.75 (-1)
410.0	3.38 (-1)	3.57 (-1)	2.76 (-1)	2.31 (-1)
420.0	3.47 (-1)	3.67 (-1)	2.84 (-1)	2.38 (-1)
430.0	3.35 (-1)	3.54 (-1)	2.74 (-1)	2.30 (-1)

TABLE 3 (continued)

λcenter (nm)	Spring	Summer	Fall	Winter
440.0	3.95 (-1)	4.18 (-1)	3.25 (-1)	2.72 (-1)
450.0	4.45 (-1)	4.70 (-1)	3.65 (-1)	3.07 (-1)
460.0	4.50 (-1)	4.75 (-1)	3.70 (-1)	3.11 (-1)
470.0	4.65 (-1)	4.91 (-1)	3.83 (-1)	3.22 (-1)
480.0	4.76 (-1)	5.03 (-1)	3.92 (-1)	3.31 (-1)
490.0	4.50 (-1)	4.76 (-1)	3.72 (-1)	3.13 (-1)
500.0	4.59 (-1)	4.85 (-1)	3.80 (-1)	3.20 (-1)
525.0	1.21	1.28	1.00	8.48 (-1)
550.0	1.26	1.33	1.05	8.83 (-1)
575.0	1.27	1.35	1.06	8.92 (-1)
600.0	1.29	1.36	1.07	9.05 (-1)
625.0	1.29	1.37	1.08	9.15 (-1)
650.0	1.30	1.38	1.09	9.24 (-1)
675.0	1.30	1.38	1.09	9.27 (-1)
700.0	1.29	1.36	1.08	9.21 (-1)
750.0	2.48	2.62	2.08	1.78
800.0	2.38	2.51	2.00	1.71

[a] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ϵ_λ in units of molar^{-1} cm^{-1} gives rate constants in units of day^{-1}.

[b] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[c] Based on the GC SOLAR program.

TABLE 4. L_λ VALUES FOR LATITUDE 30°N[a,b,c]

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	5.73 (-5)	1.09 (-4)	3.18 (-5)	6.78 (-6)
300.0	2.50 (-4)	4.11 (-4)	1.46 (-4)	4.23 (-5)
302.5	7.65 (-4)	1.14 (-3)	4.64 (-4)	1.71 (-4)
305.0	1.79 (-3)	2.46 (-3)	1.12 (-3)	4.95 (-4)
307.5	3.43 (-3)	4.45 (-3)	2.19 (-3)	1.11 (-3)
310.0	5.64 (-3)	7.02 (-3)	3.67 (-3)	2.04 (-3)
312.5	8.27 (-3)	1.00 (-2)	5.46 (-3)	3.26 (-3)
315.0	1.12 (-2)	1.32 (-2)	7.43 (-3)	4.69 (-3)
317.5	1.41 (-2)	1.64 (-2)	9.48 (-3)	6.21 (-3)
320.0	1.70 (-2)	1.95 (-2)	1.15 (-2)	7.76 (-3)
323.1	3.04 (-2)	3.46 (-2)	2.07 (-2)	1.43 (-2)
330.0	1.05 (-1)	1.18 (-1)	7.23 (-2)	5.17 (-2)
340.0	1.33 (-1)	1.48 (-1)	9.23 (-2)	6.75 (-2)
350.0	1.47 (-1)	1.63 (-1)	1.03 (-1)	7.65 (-2)
360.0	1.62 (-1)	1.80 (-1)	1.15 (-1)	8.60 (-2)
370.0	1.73 (-1)	1.91 (-1)	1.24 (-1)	9.31 (-2)
380.0	1.84 (-1)	2.04 (-1)	1.33 (-1)	1.01 (-1)
390.0	1.74 (-1)	1.93 (-1)	1.25 (-1)	9.39 (-2)
400.0	2.50 (-1)	2.77 (-1)	1.79 (-1)	1.35 (-1)
410.0	3.29 (-1)	3.64 (-1)	2.36 (-1)	1.79 (-1)
420.0	3.38 (-1)	3.74 (-1)	2.43 (-1)	1.84 (-1)
430.0	3.26 (-1)	3.61 (-1)	2.35 (-1)	1.78 (-1)

TABLE 4 (continued)

λcenter (nm)	Spring	Summer	Fall	Winter
440.0	3.86 (-1)	4.26 (-1)	2.79 (-1)	2.12 (-1)
450.0	4.34 (-1)	4.79 (-1)	3.14 (-1)	2.39 (-1)
460.0	4.39 (-1)	4.85 (-1)	3.19 (-1)	2.42 (-1)
470.0	4.54 (-1)	5.01 (-1)	3.30 (-1)	2.51 (-1)
480.0	4.65 (-1)	5.13 (-1)	3.38 (-1)	2.58 (-1)
490.0	4.40 (-1)	4.85 (-1)	3.20 (-1)	2.44 (-1)
500.0	4.49 (-1)	4.95 (-1)	3.27 (-1)	2.50 (-1)
525.0	1.18	1.31	8.67 (-1)	6.61 (-1)
550.0	1.23	1.36	9.03 (-1)	6.87 (-1)
575.0	1.24	1.37	9.11 (-1)	6.93 (-1)
600.0	1.25	1.38	9.24 (-1)	7.04 (-1)
625.0	1.26	1.39	9.34 (-1)	7.15 (-1)
650.0	1.27	1.40	9.45 (-1)	7.27 (-1)
675.0	1.28	1.40	9.48 (-1)	7.32 (-1)
700.0	1.27	1.39	9.42 (-1)	7.31 (-1)
750.0	2.44	2.67	1.82	1.41
800.0	2.34	2.57	1.75	1.37

[a] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ε_λ in units of molar^{-1} cm^{-1} gives rate constants in units of day^{-1}.

[b] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[c] Based on the GC SOLAR program.

TABLE 5. L_λ VALUES FOR LATITUDE $40^0N^{a,b,c}$

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	1.85 (-5)	6.17 (-5)	7.83 (-6)	5.49 (-7)
300.0	1.06 (-4)	2.70 (-4)	4.76 (-5)	5.13 (-6)
302.5	3.99 (-4)	8.30 (-4)	1.89 (-4)	3.02 (-5)
305.0	1.09 (-3)	1.95 (-3)	5.40 (-4)	1.19 (-4)
307.5	2.34 (-3)	3.74 (-3)	1.19 (-3)	3.38 (-4)
310.0	4.17 (-3)	6.17 (-3)	2.19 (-3)	7.53 (-4)
312.5	6.51 (-3)	9.07 (-3)	3.47 (-3)	1.39 (-3)
315.0	9.18 (-2)	1.22 (-2)	4.97 (-3)	2.22 (-3)
317.5	1.20 (-2)	1.55 (-2)	6.57 (-3)	3.19 (-3)
320.0	1.48 (-2)	1.87 (-2)	8.18 (-3)	4.23 (-3)
323.1	2.71 (-2)	3.35 (-2)	1.51 (-2)	8.25 (-3)
330.0	9.59 (-2)	1.16 (-1)	5.44 (-2)	3.16 (-2)
340.0	1.23 (-1)	1.46 (-1)	7.09 (-2)	4.31 (-2)
350.0	1.37 (-1)	1.62 (-1)	8.04 (-2)	4.98 (-2)
360.0	1.52 (-1)	1.79 (-1)	9.02 (-2)	5.68 (-2)
370.0	1.63 (-1)	1.91 (-1)	9.77 (-2)	6.22 (-2)
380.0	1.74 (-1)	2.04 (-1)	1.05 (-1)	6.78 (-2)
390.0	1.64 (-1)	1.93 (-1)	9.86 (-2)	6.33 (-2)
400.0	2.36 (-1)	2.76 (-1)	1.42 (-1)	9.11 (-2)
410.0	3.10 (-1)	3.64 (-1)	1.87 (-1)	1.20 (-1)
420.0	3.19 (-1)	3.74 (-1)	1.93 (-1)	1.24 (-1)
430.0	3.08 (-1)	3.61 (-1)	1.87 (-1)	1.20 (-1)

TABLE 5 (continued)

λcenter (nm)	Spring	Summer	Fall	Winter
440.0	3.65 (-1)	4.26 (-1)	2.22 (-1)	1.43 (-1)
450.0	4.11 (-1)	4.80 (-1)	2.51 (-1)	1.61 (-1)
460.0	4.16 (-1)	4.85 (-1)	2.54 (-1)	1.64 (-1)
470.0	4.30 (-1)	5.02 (-1)	2.63 (-1)	1.69 (-1)
480.0	4.40 (-1)	5.14 (-1)	2.70 (-1)	1.74 (-1)
490.0	4.16 (-1)	4.86 (-1)	2.56 (-1)	1.65 (-1)
500.0	4.25 (-1)	4.96 (-1)	2.62 (-1)	1.68 (-1)
525.0	1.12	1.31	6.93 (-1)	4.45 (-1)
550.0	1.16	1.36	7.21 (-1)	4.61 (-1)
575.0	1.17	1.37	7.22 (-1)	4.61 (-1)
600.0	1.18	1.38	7.39 (-1)	4.69 (-1)
625.0	1.20	1.40	7.50 (-1)	4.82 (-1)
650.0	1.21	1.41	7.62 (-1)	4.95 (-1)
675.0	1.22	1.41	7.68 (-1)	5.03 (-1)
700.0	1.21	1.40	7.66 (-1)	5.05 (-1)
750.0	2.33	2.69	1.48	9.84 (-1)
800.0	2.25	2.59	1.43	9.56 (-1)

[a] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ε_λ in the units of $molar^{-1}$ cm^{-1} gives the rate constant in units of day^{-1}.

[b] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[c] Based on the GC SOLAR program.

TABLE 6. L_λ VALUES FOR LATITUDE 50^0N[a,b,c]

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	3.61 (-6)	2.86 (-5)	9.58 (-7)	5.47 (-8)
300.0	3.05 (-5)	1.50 (-4)	8.27 (-6)	4.17 (-7)
302.5	1.54 (-4)	5.33 (-4)	4.47 (-5)	2.62 (-6)
305.0	5.24 (-4)	1.39 (-3)	1.63 (-4)	1.34 (-5)
307.0	1.32 (-3)	2.89 (-3)	4.39 (-4)	5.14 (-5)
310.0	2.66 (-3)	5.05 (-3)	9.32 (-4)	1.49 (-4)
312.5	4.53 (-3)	7.75 (-3)	1.66 (-3)	3.43 (-4)
315.0	6.82 (-3)	1.08 (-2)	2.58 (-3)	6.52 (-4)
317.5	9.34 (-3)	1.40 (-2)	3.64 (-3)	1.07 (-3)
320.0	1.19 (-2)	1.71 (-2)	4.76 (-3)	1.57 (-3)
323.1	2.25 (-2)	3.12 (-2)	9.19 (-3)	3.39 (-3)
330.0	8.26 (-2)	1.10 (-1)	3.48 (-2)	1.45 (-2)
340.0	1.09 (-1)	1.40 (-1)	4.71 (-2)	2.12 (-2)
350.0	1.22 (-2)	1.57 (-1)	5.43 (-2)	2.53 (-2)
360.0	1.36 (-1)	1.74 (-1)	6.18 (-2)	2.96 (-2)
370.0	1.47 (-1)	1.86 (-1)	6.76 (-2)	3.30 (-2)
380.0	1.57 (-1)	1.99 (-1)	7.37 (-2)	3.65 (-2)
390.0	1.48 (-1)	1.87 (-1)	6.89 (-2)	3.49 (-2)
400.0	2.12 (-1)	2.69 (-1)	9.90 (-2)	4.98 (-2)
410.0	2.80 (-1)	3.55 (-1)	1.31 (-1)	6.54 (-2)
420.0	2.89 (-1)	3.65 (-1)	1.35 (-1)	6.71 (-2)
430.0	2.79 (-1)	3.52 (-1)	1.31 (-1)	6.47 (-2)
440.0	3.31 (-1)	4.17 (-1)	1.55 (-1)	7.66 (-2)

TABLE 6 (continued)

λcenter (nm)	Spring	Summer	Fall	Winter
450.0	3.73 (-1)	4.69 (-1)	1.75 (-1)	8.62 (-2)
460.0	3.78 (-1)	4.75 (-1)	1.78 (-1)	8.74 (-2)
470.0	3.90 (-1)	4.91 (-1)	1.84 (-1)	8.95 (-2)
480.0	4.00 (-1)	5.03 (-1)	1.89 (-1)	9.15 (-2)
490.0	3.78 (-1)	4.76 (-1)	1.79 (-1)	8.62 (-2)
500.0	3.86 (-1)	4.85 (-1)	1.83 (-1)	8.77 (-2)
525.0	1.01	1.28	4.84 (-1)	2.28 (-1)
550.0	1.05	1.33	5.03 (-1)	2.32 (-1)
575.0	1.05	1.34	5.04 (-1)	2.28 (-1)
600.0	1.06	1.35	5.13 (-1)	2.32 (-1)
625.0	1.08	1.37	5.26 (-1)	2.42 (-1)
650.0	1.10	1.38	5.39 (-1)	2.53 (-1)
675.0	1.11	1.39	5.47 (-1)	2.61 (-1)
700.0	1.11	1.38	5.49 (-1)	2.66 (-1)
750.0	2.15	2.66	1.07	5.22 (-1)
800.0	2.08	2.57	1.04	5.11 (-1)

[a] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ε_λ in the units of molar^{-1} cm^{-1} gives the rate constant in units of day^{-1}.

[b] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[c] Based on the GC SOLAR program.

B. Appendix B. Illustrative Examples

1. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Rate Constant and Minimum Half-life in Sunlight

a. Illustrative Example 1

A neutral organic chemical A was dissolved in water at a concentration of 1.00×10^{-3} M. UV-visible absorption spectra were obtained in a 10.0 cm quartz absorption cell and no absorbance was detected above the baseline in the region 290 nm and greater (i.e., $A_\lambda = 0$ for $\lambda > 290$ nm). Since $A_\lambda = 0$, then $\varepsilon_\lambda^c = 0$ (equation 15). Using this result in equation 7, it is found that $(k_{pE})_{max.} = 0$, indicating that no direct photolysis can take place in sunlight at any latitude or season of the year. This example illustrates the principle of the Grotthus-Draper law, the first law of photochemistry. That is, in order for direct photolysis to take place in sunlight, the chemical must absorb sunlight in the region $\lambda > 290$ nm.

b. Illustrative Example 2

Consider a plant located in Columbus, Georgia on the Chattahoochee River which produces an organic chemical B which is not an acid or a base. The waste effluent passes through a primary and secondary treatment plant and is then discharged directly into the river. The plant produces chemical B continuously every day of the year. The plant is located at 32.5° north latitude. Estimate the maximum sunlight direct photolysis rate constant and the corresponding minimum half-life for this chemical in the river for the winter and summer seasons under clear skies.

Laboratory Experiments, Data, and Calculations

The water solubility of chemical B is 1.00×10^{-3} M at 25°C. Chemical B was dissolved directly in water and a 1.00×10^{-4} molar solution was prepared at 25°C. The uv-visible absorption spectra were obtained according to the Tier 1 Procedure in a 10.0 cm quartz absorption cell in duplicate. Using the wavelength interval range (from Table 1), the average absorbance of the duplicate runs at λcenter was obtained and the results are summarized in Table 7.

TABLE 7. SUMMARY OF PHOTOLYSIS DATA FOR CHEMICAL B

A. SPECTRAL DATA

λcenter(nm)	A_λ	ε_λ^c (M^{-1} cm^{-1})
297.5	1.684	1684
300.0	1.434	1434
302.5	1.221	1221
305.0	0.919	919
307.5	0.742	742
310.0	0.208	208
312.5	0.138	138
315.0	0.094	94
317.5	0.057	57
320.0	0.009	9
323.1	0.002	2
330.0	0.000	0

TABLE 7 (continued)

B. PHOTOLYSIS DATA

	Summer		Winter	
λcenter(nm)	L_λ^*	$\varepsilon_\lambda^c L_\lambda$ (day^{-1})	L_λ^*	$\varepsilon_\lambda^c L_\lambda$ (day^{-1})
297.5	1.09 (-4)	0.18	6.78 (-6)	0.01
300.0	4.11 (-4)	0.59	4.23 (-5)	0.06
302.5	1.14 (-3)	1.39	1.71 (-4)	0.21
305.0	2.46 (-3)	2.26	4.95 (-4)	0.45
307.5	4.45 (-3)	3.30	1.11 (-3)	0.82
310.0	7.02 (-3)	1.46	2.04 (-3)	0.42
312.5	1.00 (-2)	1.38	3.26 (-3)	0.45
315.0	1.32 (-2)	1.24	4.69 (-3)	0.44
317.5	1.64 (-2)	0.94	6.21 (-3)	0.35
320.0	1.95 (-2)	0.18	7.76 (-3)	0.07
323.1	3.46 (-2)	0.07	1.43 (-2)	0.03
330.0	1.18 (-1)	0.00	5.17 (-2)	0.00
		$\sum \varepsilon_\lambda^c L_\lambda$ = 12.99		$\sum \varepsilon_\lambda^c L_\lambda$ = 3.31

---

*The units of L_λ are in 10^{-3} einsteins cm^{-2} day^{-1}. The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

From the above data and equation 15, the average molar absorptivity is

$$\varepsilon_{\lambda} = 1000\ A_{\lambda} \quad . \tag{18}$$

From the average A_{λ} value at λ center, the average molar absorptivity can be obtained from equation 18 and the results are summarized in Table 7. Since the plant is located at 32.5° north latitude, the closest L_{λ} values are at 30° north latitude. These values are obtained from Table 4 and are summarized in Table 7 for the summer and winter seasons. Using the data from Table 7 and equations 7 and 8, the following results are obtained.

Summer	Winter
$(k_{pE})\text{max.} = \sum \varepsilon_{\lambda}^{c} L_{\lambda} = 13.0\ \text{day}^{-1}$	$(k_{pE})\text{max.} = \sum \varepsilon_{\lambda}^{c} L_{\lambda} = 3.31\ \text{day}^{-1}$
$(t_{1/2E})\text{min.} = 0.053\ \text{day}$	$(t_{1/2E})\text{min.} = 0.21\ \text{day}$

Since the chemical transforms rapidly for the summer and winter seasons, it is necessary to carry out Tier 2 experiments to more accurately define direct photolysis rates in aqueous media as a function of the season of the year.

2. Tier 2 Phase 1: Aqueous Photolysis in Sunlight

a. Illustrative Example 3

Consider the same scenario as described in illustrative example 2, Section V.B.1.b. Using the Tier 2, Phase 1 Procedure, carry out experiments to estimate the rate of direct photolysis and half-life in aqueous solution in the spring for water bodies.

Photolysis Experiments and Calculations

Since chemical B absorbs appreciably below 340 nm, 11 mm i.d. quartz tubes were used (note: this tube has an approximate pathlength of 1 cm). Chemical B was dissolved directly in pure water and a 1.00×10^{-5} molar solution was prepared at 25°C. Since the water solubility is 1.00×10^{-3} M at 25°C, this sample solution was well below one-half its water solubility. The uv spectrum of this solution in a one cm absorption cell indicated that A_λ was less than 0.05 at $\lambda \geq 290$ nm. Hence, under these conditions, first-order kinetics are applicable.

A series of quartz tubes were filled with this aqueous solution, sealed, and photolysis experiments were carried out in sunlight according to the appropriate procedure described in Section II.B.3.a. The experiments were started at noon (1200 hours) on May 8, 1982. The weather conditions are summarized for this period of time and the concentration data given represent the mean of duplicate determinations.

(1) May 2, 1982: at t = 0 (noon - 1200 hours)
$C_o = 1.00 \times 10^{-5}$ M.

(2) May 2, 1982: Noon to sunset - clear and sunny.

(3) May 3, 1982: Noon (1200 hours), $C_t = 0.840 \times 10^{-5}$ M.

(4) May 3, 1982: at 1400 hours the weather conditions were cloudy with rain. The rain and cloudy weather continued until 2200 hours.

(5) From sunrise, May 4, 1982 through 1200 hours May 8, 1982, the weather was clear and sunny. At 1200 hours, May 8, 1982, analysis of the samples gave an average concentration of $C_t = 0.400 \times 10^{-5}$ M. Since 60% of chemical B transformed, the photolysis experiments were terminated and the control samples were analyzed. The average concentration of the control samples was 0.997×10^{-5} M which was essentially the same as C_o. Hence, no adventitious processes occurred and the loss of chemical was only due to sunlight photolysis.

Listed in Table 8 are the times of sunrise and sunset for the dates sunlight photolysis experiments were carried out along with the total number of hours of sunlight.

88

TABLE 8. SUMMARY OF TIMES FOR SUNRISE AND SUNSET FOR THE PERIOD MAY 2-8, 1982

Date (1982)	Sunrise (AM)	Sunset (PM)	Total Sunlight Hours
5/2	0600	2010	14.2
5/3	0559	2011	14.2
5/4	0558	2012	14.2
5/5	0557	2013	14.3
5/6	0556	2014	14.3
5/7	0555	2015	14.3
5/8	0554	2016	14.4

The following data summarizes the dates photolyzed, the times exposed to sunlight, the total sunlight photolysis time for each date in days, the total number of days of sunlight photolysis, and the calculation of k_p^c and $t_{1/2}$.

Date	Times Photolyzed		Sunlight Photolysis Time for Each Date (days)
5/2	1200 hrs. to 2010 hrs.	(8.2/14.2)	0.58
5/3	0559 hrs. to 1200 hrs.	(6.0/14.2)	0.42
5/3	1200 hrs. to 1400 hrs.	(2.0/14.2)	0.14
5/4	0558 hrs. to 2012 hrs.		1.00
5/5	0557 hrs. to 2013 hrs.		1.00
5/6	0556 hrs. to 2014 hrs.		1.00
5/7	0555 hrs. to 2015 hrs.		1.00
5/8	0554 hrs. to 1200 hrs.	(6.1/14.4)	0.42
			total 5.56 days

$$t = 5.6 \text{ days};\ C_o = 1.00 \times 10^{-5};\ C_t = 0.400 \times 10^{-5}$$

$$\ln(C_o/C_t) = k_p^c t$$

$$k_p^c = (1/t)\ \ln(C_o/C_t) = (1/5.6)\ln(1.00 \times 10^{-5}/0.400 \times 10^{-5})$$

$$k_p^c = 0.16 \text{ days}^{-1}$$

$$t_{1/2} = 0.693/0.16 \text{ days}^{-1} = 4.3 \text{ days}$$

Therefore, the rate constant for direct photolysis of chemical B in tubes in pure water is 0.16 days^{-1} and the corresponding half-life is 4.3 days for the period of photolysis May 2-8, 1982 at 32.5° north latitude. Using equation 13, the direct photolysis rate constant (k_{pE}) for water bodies is 0.073 days^{-1} and the corresponding half-life ($t_{1/2E}$) is 9.5 days.

3. Tier 2, Phase 2: Aqueous Photolysis in Sunlight

a. Illustrative Example 4

Consider the same scenario as described in illustrative examples 2 and 3. Using the Tier 2, Phase 2, Procedure, carry out experiments to determine the sunlight reaction quantum yield and estimate the rate constant for direct photolysis in aqueous solution and the half-life for water bodies and clear sky conditions for the summer and winter seasons.

90

Photolysis Experiments and Calculations

The sunlight photolysis experiments were carried out in the beginning of May, 1982 at 32.5° north latitude.

(1) Preparation of the Actinometer Solution

The results from the Tier 2, Phase 1, experiments indicated that k_p^c for the test chemical was 0.16 days^{-1}. Since the experiments were carried out in early May at 32.5° north latitude, the value of k_a^a was chosen from Table 2 which corresponds to the spring season and at 30° north latitude; and the value is 483 days^{-1}. Using equation 11, the molar concentration of pyridine required to adjust the actinometer rate to match the rate of disappearance of the test chemical is

$$[PYR] = 26.9\ (0.16/483) = 8.91 \times 10^{-3} \text{ molar.}$$

Using this concentration of pyridine, an actinometer solution was prepared according to the procedure described in Section II.B.h. The quantum yield for this actinometer is calculated using equation 9.

$$\phi_E^a = 0.0169[PYR] = 0.0169(8.91 \times 10^{-3}) = 1.51 \times 10^{-4}$$

Procedures for Tier 2, Phase 2 experiments (Section II.B.3.b) were followed and sunlight experiments were initiated at 1200 hours on May 9, 1982. The mean initial concentration of test chemical was 1.00×10^{-5} molar and the mean initial concentration of PNAP was 1.00×10^{-5} molar. Samples of the chemical and actinometer and the controls were analyzed in triplicate periodically at 1200 hours on May 10, 11, 13, 15, and 16. On May 16, the photolysis experiments were terminated. The mean concentrations of all samples are summarized below.

Date	Conc. of Chem. (M)	Conc. of Act. (M)	Conc. of Chem. Control (M)	Conc. of Act. Control (M)
May 9	1.00×10^{-5}	1.00×10^{-5}	1.00×10^{-5}	1.00×10^{-5}
May 10	0.820×10^{-5}	0.855×10^{-5}	0.997×10^{-5}	1.00×10^{-5}
May 11	0.654×10^{-5}	0.710×10^{-5}	1.00×10^{-5}	0.997×10^{-5}
May 13	0.440×10^{-5}	0.515×10^{-5}	0.996×10^{-5}	0.999×10^{-5}
May 15	0.299×10^{-5}	0.383×10^{-5}	0.999×10^{-5}	0.998×10^{-5}
May 16	0.233×10^{-5}	0.304×10^{-5}	0.997×10^{-5}	0.996×10^{-5}

Since no significant loss of PNAP or test chemical was observed in the control samples, no adventitious processes occurred and the loss of test chemical and PNAP was only due to sunlight photolysis.

Using the above data, $\ln(C_o/C_t)$ for the test chemical and actinometer can be calculated and the results are summarized below.

	Chemical		Actinometer	
t (days)	$C_t \times 10^5$ (M)	$\ln (C_o/C_t)^c$	$C_t \times 10^5$ (M)	$\ln (C_o/C_t)^a$
0	1.00	0.000	1.00	0.000
1	0.820	0.199	0.855	0.157
2	0.654	0.425	0.710	0.343
4	0.440	0.821	0.515	0.664
6	0.299	1.21	0.383	0.960
7	0.233	1.46	0.304	1.19

The ratio of the rate constants, k_p^c/k_p^a, is defined by equation 12

$$\ln(C_o/C_t)^c = (k_p^c/k_p^a)\ \ln(C_o/C_t)^a \quad . \qquad (12)$$

Using all the data (including the time point t = 0) and linear regression analysis, the slope is found to be 1.237 with a correlation coefficient of 0.9998. Therefore

$$(k_p^c/k_p^a) = 1.24 \quad .$$

Using the molar absorptivities obtained in example 2, Section V.B.1.b., and the L_λ values for spring at 30° north latitude, Table 4, the value of $\sum \varepsilon_\lambda^c L_\lambda$ can be calculated as follows:

λcenter(nm)	$\varepsilon_\lambda^c(M^{-1}cm^{-1})$	L_λ^*	$\varepsilon_\lambda^c L_\lambda$ (days^{-1})
297.5	1684	5.73 (-5)	0.10
300.0	1434	2.50 (-4)	0.36
302.5	1221	7.65 (-4)	0.93
305.0	919	1.79 (-3)	1.65
307.5	742	3.43 (-3)	2.55
310.0	208	5.64 (-3)	1.17
312.5	138	8.27 (-3)	1.14
315.0	94	1.12 (-2)	1.05
317.5	57	1.41 (-2)	0.80
320.0	9	1.70 (-2)	0.15
323.1	2	3.04 (-2)	0.06
330.0	0	1.05 (-1)	0.00

* The units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}.

$$\sum \varepsilon_\lambda^c L_\lambda = 9.96 \text{ days}^{-1}.$$

For this experiment, $k_a^a(\sum = \varepsilon_\lambda^a L_\lambda)$ is 483 days^{-1} (Table 2). All the pertinent data are summarized below:

$$k_p^c/k_p^a = 1.24; \quad \sum \varepsilon_\lambda^c L_\lambda = 9.96 \text{ days}^{-1};$$
$$\sum \varepsilon_\lambda^a L_\lambda = 483 \text{ days}^{-1}; \quad \phi_E^a = 1.51 \times 10^{-4} .$$

Substituting these results into equation 10 yields

$$\phi_E^c = (1.24)(483/9.96)(1.51 \times 10^{-4})$$

$$\phi_E^c = 9.08 \times 10^{-3} .$$

The rate constants for direct photolysis of test chemical in aqueous media and the half-life for water bodies and clear sky conditions for the winter and summer seasons can be calculated as follows: The values of $\sum \varepsilon_{\lambda}^{C} L_{\lambda}$ have been calculated from example 1, Section V.B.1.b. For summer $\sum \varepsilon_{\lambda}^{C} L_{\lambda} = 13.0$ days^{-1}; for winter $\sum \varepsilon_{\lambda}^{C} L_{\lambda} = 3.31$ days^{-1}. The reaction quantum yield for the chemical is 9.08×10^{-3}. Using these data in equation 4 yields

summer: $k_{pE} = 9.08 \times 10^{-3}\ (13.0) = 0.118\ \text{days}^{-1}$

winter: $k_{pE} = 9.08 \times 10^{-3}\ (3.31) = 0.0301\ \text{days}^{-1}$.

These values can be substituted into equation 2 to obtain the half-lives for these two seasons.

summer: $t_{1/2E} = (0.693/0.118) = 5.9$ days

winter: $t_{1/2E} = (0.693/0.0301) = 23$ days.

CS-6000
October, 1984

PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

Contents

Page

I. NEED FOR THE TEST........ 1

II. SCIENTIFIC ASPECTS........ 2

- A. Rationale for the Selection of the Test Method..... 2
  - 1. Historical Discussion........ 2
  - 2. Selection of the Test Method........ 6
- B. Development of the Test Method........ 9
  - 1. Theoretical Aspects........ 9
  - 2. Tier 1 Test: UV-Visible Absorption Spectra-Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-life in Sunlight........ 12
    - a. Determination of Molar Absorptivity........ 13
    - b. Solar Irradiance........ 15
    - c. Estimation of the Maximum Direct Photolysis Rate Constant and the Corresponding Minimum Half-life........ 15
  - 3. Tier 2 Test: Aqueous Photolysis in Sunlight... 16
    - a. Phase 1 Test........ 16
    - b. Phase 2 Test........ 16
      - i. Introduction........ 16
      - ii. Development of a Sunlight Actinometer........ 18
      - iii. Geometry of the Reaction Vessel...... 22
      - iv. Phase 2 Test Method........ 23
  - 4. Summary........ 27
- C. Applicability and Specificity........ 28
- D. Rationale for the Selection of the Test Conditions........ 30
  - 1. Special Laboratory Equipment........ 30
  - 2. Purity of Water........ 31
  - 3. Sterilization........ 32
  - 4. Concentration of Solution........ 32
  - 5. pH Effects........ 33
  - 6. Control Solution........ 34
  - 7. Absorption Spectrum as a Criterion for Performing the Aqueous Photolysis Test....... 35

8. Sunlight Actinometer.......................... 35
9. Solar Irradiance Data.......................... 38
10. Geometry of the Reaction Vessel................ 43
11. Chemical Analysis of Solutions................. 43
12. Outdoor Experimental Conditions................ 44

III. REFERENCES..................................... 46

IV. APPENDIX: GLOSSARY OF IMPORTANT SYMBOLS......... 49

98

PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

I. NEED FOR THE TEST

The majority of the earth's surface is covered by water in the form of oceans, seas, rivers, lakes, streams, or ponds. As a result, chemicals are likely to enter aqueous media and could then undergo transformation via direct aqueous photolysis.

Direct aqueous photolysis represents the transformation of a chemical substance by direct absorption of radiant energy (sunlight) into new chemicals different from their precursors. Chemical substances which are present in aqueous media photolyze at different rates depending upon the solar irradiance, the chemical substance's molar absorptivity at each wavelength of solar radiation, and its reaction quantum yield at the wavelengths of concern. Chemical substances which photolyze rapidly under environmental conditions have relatively short lifetimes in the environment. Consequently, the chemical fate assessment may focus on the transformation products to a greater extent than on the parent compound. On the other hand, if the chemical substance is resistant to photolysis as well as to all the other possible transformation processes, the assessment should focus on the parent chemical.

A cost-effective aqueous photolysis test is needed to assess quantitatively the transformation of chemical substances in sunlight. The importance of direct photolysis in sunlight as a transformation process for chemical substances in aqueous media in the environment can be determined quantitatively from data on photolysis rate constants and half-lives.

This aqueous photolysis test represents a screening test to allow one to determine how rapidly direct photolysis will take place in aqueous media under certain environmental conditions. If the photolysis test data indicate that photolysis is a relatively important transformation process and the initial risk assessment indicates that there is a threat to the health of humans and/or to the environment, then upper-tier tests may be required to obtain more precise aqueous photolysis data over a wide range of environmental conditions. These upper-tier tests will also be concerned with determining the identity and fate of the transformation products.

II. SCIENTIFIC ASPECTS

A. Rationale for the Selection of the Test Method

1. Historical Discussion

The scientific literature contains a number of publications dealing with the photolysis in solution of various chemical substances. Unfortunately, for one or more reasons, most of the methods and data contained in the literature are of little or no use in determining aqueous photolysis rate constants and half-lives. Reasons for this include: (1) Many of the publications deal primarily with the mechanisms and products from direct photolysis reactions rather than photolysis rates. (2) The publications give no quantitative data on the rates of photolysis under environmental conditions; i.e., many researchers used sources of light which do not simulate sunlight or performed experiments in solvents other than air-saturated water. (3) Some studies are not based upon the fundamental laws of photochemistry. (4) Many publications report the effects of certain sensitizers which are environmentally irrelevant, or the effect of other chemicals present in solution, on the chemical substance being studied. (5) Many of the studies suffer from poor experimental design, overlooking the controls necessary to make sure that only photolysis and not biodegradation, volatility, or other competing processes are taking place to remove or transform the chemical substance. The following paragraphs cite a few representative examples which illustrate the points mentioned above, as well as the publication which forms the basis for this Test Guideline.

A paper by Grunwell and Erickson (1973) deals with the photochemistry of parathion. This report is of little relevance in the evaluation of photolysis rates of chemical substances for several reasons. The main emphasis of this work is on the identification of major products formed by the photolysis of parathion rather than in the measurement of rate constants and half-lives. Photolysis experiments were performed at three wavelengths (254, 300, and 350 nm), of which only the last two are environmentally relevant. The chemical was dissolved in solutions of 20 percent water and either 80 percent ethanol or tetrahydrofuran (by weight), neither of which is environmentally relevant.

A paper by Langford et al. (1973) on the photolysis of nitrilotriacetic acid claims environmental relevance by working under environmental conditions. Radiation of 350 nm as well as sunlight were used. Pure water and river water were used as solvents. The authors presented no quantitative data on the rate of photolysis. Detailed quantitative measurement of the photolysis rate constant and half-life, along with adequate controls, would be necessary to make this research useful for the purposes of this Test Guideline.

Benson et al. (1971) photolyzed chlordane with both mercury arc radiation and sunlight. Acetone was used as both a solvent and photosensitizer. Sunlight photolysis was done only on the pure compound applied as a thin layer under quartz glass. The main emphasis of this work was to look at the chemical structure of the reaction products. Because rate constants were not measured, acetone was used as both a solvent and sensitizer, and a mercury arc lamp was used as radiation source, their procedures have minimal applicability as a test method to screen for environmental photolysis rates.

Su and Zabik (1972) studied the photochemistry of arylamidine derivatives in distilled and natural water. A high pressure mercury arc filtered to remove radiation below 286 nm was used. Products of the reaction were determined but no kinetic studies were performed and no rate constants or half-lives were reported.

Mancini (1978) presented a theoretical framework for the first-order photodecomposition of picloram in aqueous solution and tested the framework using some experimental data on the photolysis of picloram in sunlight. In reality, the Mancini framework is only an empirical approach describing the rate of photolysis of picloram in sunlight.

Zepp and Cline (1977) published a paper on direct photolysis in aqueous environments with equations for the determination of direct photolysis rate constants in sunlight. This paper avoids the problems illustrated above and serves as the starting point for the development of a test method for direct photolysis in aqueous solution in sunlight. These equations translate readily obtained laboratory data into rate constants and half-lives for sunlight photolysis. Photolysis half-lives can be calculated as a function of season, latitude, time of day, depth in water bodies, and thickness of the atmospheric ozone layer. A number of published papers concerning the photolysis of chemicals in sunlight have verified this method. (e.g., Wolfe et al. 1976, Smith et al. 1977, 1978, Zepp et al. 1975, 1976).

During the past few years mathematical models have become available for predicting the behavior of chemicals in the environment. A number of efforts have been made to validate the models through studies of the fate of pollutants under various field conditions or in outdoor ecosystems that have known inputs of the chemical, controlled flow conditions, etc. Several of these field studies confirmed that direct photochemical reactions dominate the behavior of certain pollutants in natural waters and the equations used in the model do a reasonably good job of predicting rates in water bodies based on the work of Zepp and Cline (1977). The following citations illustrate some of these research efforts [Broderius and Smith (1980), Carey and Fox (1981), Crossland and Wolf (1984), Mabey et al. (1983), Simmons and Zepp (1984), Wolfe et al. (1982), Zepp (1983), and Zepp (1982a)].

2. Selection of the Test Method

The methods in this Test Guideline were developed from a thorough review of the research literature on the experimental determination of aqueous photolysis rate constants, the principles outlined by Zepp and Cline (1977), Zepp (1978), the method developed by the U.S. Environmental Protection Agency, Office of Toxic Substances [USEPA (1979)], and by the research carried out by SRI International [Mill et al. (1981, 1982a, 1982b) and Dulin and Mill (1982)].

The proposed test method for the measurement of direct sunlight photolysis of a chemical substance in aqueous solution is based upon four fundamental criteria. These criteria are:

(1) The test method should be based upon the fundamentals of photochemistry. (2) The test method should yield quantitative data on direct photolysis rates of chemical substances in aqueous media. (3) Sunlight should be used as the irradiation source because of its obvious environmental relevance as well as its low cost in comparison to artificial light sources (Zepp 1982). (4) The test method should be designed to account for transformation or chemical losses by mechanisms other than photolysis. For example, the experiments should be designed to eliminate or minimize biodegradation and volatilization and to account for hydrolysis as a factor in the estimation of test substance losses.

A simple first-tier screening test is used to estimate the direct aqueous photolysis rate constant and half-life in sunlight as a function of latitude and season of the year. An upper limit for this rate of photolysis can be obtained by assuming that photolytic transformation occurs with a reaction quantum yield of unity and by equating the rate of reaction with the rate of absorption of light. The latter rate, which represents the maximum rate constant, can be estimated as a function of latitude and season of the year by combining ultraviolet-visible absorption spectral data with the appropriate solar irradiance data. The maximum rate constant can then be used to obtain the corresponding minimum half-life for photolysis in water in sunlight. If these data indicate that aqueous photolysis is an important transformation process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.) then additional tests (i.e., upper-tier tests) should be carried out to determine environmentally relevant rate constants and half-lives.

A second-tier aqueous photolysis screening test is used to determine rate constants and half-lives in the presence of sunlight. This method is divided into two phases. In phase one, the test chemical is photolyzed in sunlight in order to obtain an approximate rate constant [USEPA (1979)]. However, this method only gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis. In phase two, a standard actinometer is used to overcome this deficiency [Mill et al. (1981, 1982b) and Dulin and Mill (1982)]. The rate constant of the standard actinometer can be adjusted so that its half-life can range from a few hours to several weeks. By using the data from phase one, the rate constant of the standard actinometer can be adjusted to match the approximate rate constant of the test chemical. Then, by simultaneously photolyzing the test chemical and the standard actinometer in sunlight, the sunlight reaction quantum yield of the test chemical can be determined. Combining this reaction quantum yield with the test chemical molar absorptivity data from the tier-one test and the appropriate solar irradiance data in water, environmentally relevant rate constants (i.e., for water bodies under clear sky conditions) can be calculated as a function of latitude and season of the year. These rate constants can then be used to determine the corresponding half-lives for photolysis of a test chemical in sunlight in aqueous media. These rate constants are in a form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

B. Development of the Test Method

1. Theoretical Aspects

The theory of the method of Zepp and Cline (1977) is briefly discussed to show that the proposed test method is based upon the fundamental criteria given in Section II.A.2. These discussions lay the foundation for the proposed method, show how the method can be used to obtain direct sunlight photolysis rate constants and half-lives, and indicate what test conditions must be standardized in order to obtain meaningful aqueous photolysis rate data.

Zepp and Cline (1977) published a paper on the rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water body. It has been shown that in dilute solution (i.e., the absorbance of a chemical is less than 0.02 in the reaction cell at all wavelengths greater than 290 nm), the kinetic expression for direct photolysis of a chemical at a molar concentration C is

$$-\frac{dC}{dt} = \phi_E k_a C = k_{pE} C \qquad (1)$$

$$k_{pE} = \phi_E k_a \quad , \qquad (2)$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, and $k_a = \sum k_{a\lambda}$, the sum of $k_{a\lambda}$ values of all wavelengths of sunlight that are absorbed by the chemical (i.e., the light absorption rate constant). The term k_{pE} represents the photolysis rate constant for water bodies in sunlight in the units of reciprocal time. Integrating equation 1 yields

$$\ln(C_o/C_t) = k_{pE} t \quad , \qquad (3)$$

where C_t is the molar concentration of chemical at time t during photolysis and C_o is the initial molar concentration. By measuring the concentration of chemical as a function of the time t during photolysis in sunlight, k_{pE} can be determined using equation 3. In addition, equation (3) can be solved for the condition $C_t = C_o/2$ and the half-life of the chemical is given by

$$t_{1/2E} = \frac{0.693}{k_{pE}} \quad . \qquad (4)$$

Furthermore, under the same conditions cited above [i.e., for homogeneous chemical solution with absorbance less than 0.02* in a reaction cell at all wavelengths greater than 290 nm and at shallow depths (less than 0.5 m)], the first-order direct photolysis rate constant, k_{pE}, is

$$k_{pE} = \phi_E \sum \varepsilon_\lambda L_\lambda \quad , \tag{5}$$

where ϕ_E is the reaction quantum yield which is independent of the wavelength, ε_λ is the molar absorptivity in the units molar^{-1} cm^{-1}, and L_λ is the solar irradiance in water in the units 10^{-3} einsteins cm^{-2} day^{-1} [Mill et al. (1981, 1982a, 1982b)]. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this guideline measures rate constants in tubes (e.g., 13 x 100 mm) and the rate is faster in tubes. This is discussed in more detail in Section II.B.3.b.iii. Thus, equations 3 and 4 have to be modified to take this into account. For simplicity, the following nomenclature is used. For water bodies, the rate constant is designated as k_{pE} with the subscript E designating rates in the environment in water bodies. For tubes, the rate constant is designated as k_p. The corresponding half-lives for water bodies and tubes are $t_{1/2E}$ and $t_{1/2}$, respectively. Thus, for tubes, equations 3 and 4 can be written as

---

*At an absorbance of 0.05, equation (5) is in error by only 11%. This is an acceptable error limit for a screening test and an absorbance of 0.05 has been adopted for use in this Test Guideline.

$$\ln(C_o/C_t) = k_p t \tag{6}$$

$$t_{1/2} = \frac{0.693}{k_p} \quad . \tag{7}$$

2. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-life in Sunlight

A simple first-tier screening test has been developed using equation 5. As an approximation, it is assumed that the reaction quantum yield ϕ_E is equal to one, the maximum value*. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 5 becomes

---

*It is possible that under certain circumstances the reaction quantum yield can be greater than 1. For example, this happens when a chain reaction occurs after the absorption of a quantum of light. However, this rarely occurs in dilute aqueous solution.

$$(k_{pE})_{max.} = \sum \varepsilon_\lambda L_\lambda \quad . \tag{8}$$

Substituting equation 8 in equation 4, the lower limit for the half-life is then given by

$$(t_{1/2E})_{min.} = \frac{0.693}{(k_{pE})_{max.}} = \frac{0.693}{\sum \varepsilon_\lambda L_\lambda} \quad . \tag{9}$$

In order to estimate $(k_{pE})_{max.}$, it is necessary to obtain data on the molar absorptivities of the test chemical and the solar irradiance (equation 8). These two parameters can be obtained in the following way.

a. Determination of Molar Absorptivity

The molar absorptivity of a solute (test chemical) can be obtained from the Beer-Lambert law and the ultraviolet-visible absorption spectrum. The Beer-Lambert law states that for an absorbing solute present in water, the decrease in intensity of light with thickness $(-dI/d\ell)_\lambda$, at a fixed wavelength λ, is proportional to the intensity I and the concentration of the solute C in the medium [Prutton and Maron (1951)]. Therefore,

$$-(dI/d\ell)_\lambda = \varepsilon'_\lambda CI \quad , \tag{10}$$

where ε'_λ is the proportionality constant. Equation 10 can be easily integrated to give the standard Beer-Lambert equation

$$\log_{10}(I_o/I)_\lambda = \varepsilon_\lambda C\ell \quad , \tag{11}$$

where ℓ is the thickness of the solution or pathlength of the cell in cm, I_o is the intensity of light at $\ell = 0$, I is the intensity of light at the thickness ℓ, C is the molar concentration, and ε_λ is the molar absorptivity in the units $\text{molar}^{-1}\ \text{cm}^{-1}$.

Since A_λ, the absorbance, is equal to $\log_{10}(I_o/I)_\lambda$, equation 11 becomes

$$A_\lambda = \varepsilon_\lambda C\ell \quad . \tag{12}$$

The absorbance A_λ can be obtained from the ultraviolet-visible absorption spectrum of the test chemical in aqueous solution by the methods outlined in Test Guideline CG-1050. These data will be used in equation 12 to calculate molar absorptivities for the appropriate wavelength intervals and wavelength centers where the test chemical absorbs light. These wavelength intervals and wavelength centers are listed in Table 1 of the Test Guideline (CG-6000). The method outlined in the Test Guideline CG-1050 is the standard procedure used by all laboratories for determining the molar absorptivity of a chemical in solution.

b. Solar Irradiance

The solar irradiance in water, L_λ, is related to the sunlight intensity in water and is proportional to the average light flux that is available to cause photoreaction in the wavelength interval centered at λ over a 24-hour day. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year. The solar irradiance is discussed in more detail in Section II.C.9. and the values are listed in Tables 3-6 in the Test Guideline (CG-6000) as a function of latitude and season of the year.

c. Estimation of the Maximum Direct Photolysis Rate Constant and the Corresponding Minimum Half-Life

The maximum direct photolysis rate constant is defined by equation 8. The L_λ values are chosen from Tables 3-6 in the Test Guideline (CG-6000) for the appropriate latitude and season of the year. The molar absorptivity is determined according to Section II.B.2.a. The product of ε_λ and L_λ is calculated at λcenter for each wavelength interval where ε_λ has a non-zero value. Then the products of $\varepsilon_\lambda L_\lambda$ are summed over all wavelength intervals for λcenter $>$ 299 nm. This sum is $\sum \varepsilon_\lambda L_\lambda$ and is equal to $(k_{pE})_{max.}$. $(k_{pE})_{max.}$ is substituted into equation 9 to obtain the minimum half-life.

3. Tier 2 Test: Aqueous Photolysis in Sunlight

a. Phase 1 Test

The phase one aqueous photolysis test is based on the method developed by the U.S. Environmental Protection Agency, Office of Toxic Substance [USEPA (1979)] using equations 6 and 7. By measuring the concentration of chemical as a function of time t during photolysis in sunlight, k_p^c can be determined. This rate constant is valid for the latitude and season of the year in which the photolysis experiments were carried out. The half-life can then be calculated using k_p^c in equation 7. This method only gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis. This data will be used in the phase two aqueous photolysis screening test.

b. Phase 2 Test

The phase two aqueous photolysis sunlight photolysis test method is based on the research carried out at SRI International [Mill et al. (1981, 1982b) and Dulin and Mill (1982)].

i. Introduction

The phase one test method has a limitation since it does not measure sunlight intensities incident on the sample during photolysis. SRI carried out a study on the use of insolation methods for measuring solar intensity (e.g., radiometry, photometry, and actinometry). The most suitable method for measuring sunlight intensity was found to be actinometry. Radiometry is used to measure total solar flux, but because radiometry involves energy rather than photon flux, it is of limited value for estimating photoselective photochemical reactions. Photometers, based on photocells and photomultipliers, have several advantages over radiometers, the most important being that they are sensitive to photon flux rather than energy flux. However, sensitive photometers, using digital pulse-counting methods, are relatively expensive and fragile. Chemical actinometry offers advantages over instrumental methods. Actinometry conforms to the geometry of the reaction vessel and measures the actinic flux under known sensitivity conditions. Thus, in general, actinometry, to measure solar intensity, is simple to use in aqueous photolysis experiments. The main complication in using actinometry in outdoor experiments is that the sun is not a monochromatic source, and the absorption of light by the actinometer and chemical rarely match. Therefore, a series of actinometers would be needed to match the light absorption of the test chemical in the wavelength region $\lambda > 290$ nm. Fortunately, the most important wavelength region for sunlight photolysis is at 290-400 nm, where the high energy sunlight is concentrated, and a large number of chemicals absorb light in this region. Therefore, the most useful actinometer is one which absorbs light in the region 290-400 nm.

ii. Development of a Sunlight Actinometer

Listed below are several criteria for chemical and spectral properties which should be given high priority in selecting sunlight actinometers. The ideal actinometer should:

(1) absorb light moderately and uniformly in the solar region but be stable in room light;

(2) have a reaction quantum yield, ϕ, which is wavelength independent;

(3) have a reaction quantum yield that is independent of dissolved oxygen and concentration of the actinometer;

(4) have an adjustable half-life ranging from several hours to 90 days;

(5) have photolysis products that do not interfere with photolysis, either chemically or spectrally;

(6) have simple, accurate, analytical methods available for its analysis;

(7) be very stable in water in the dark;

(8) be moderately soluble in water and relatively nonvolatile; and

(9) have little or no temperature dependence of ϕ.

Based on a review of the photochemical literature, several hundred examples of photochemical reactions were found, many of which might fulfill some of the criteria established for the ideal actinometer. To simplify the task, the review was restricted to photoreactions that are bimolecular and are therefore amenable to changes in the rate (i.e., to changes in the value of ϕ) with changes in concentration of one photoinert reactant. Nucleophilic substitutions constituted one such class.

The most successful actinometer developed to date with a variable reaction quantum yield is the system composed of p-nitroacetophenone (PNAP) and pyridine (PYR) [Mill et al. (1981, 1982b) and Dulin and Mill (1982)]. Laboratory experiments at 313 nm and 25°C indicated that the reaction quantum yield for transformation of PNAP depended linearly on the molar concentration of pyridine, at a fixed concentration of PNAP. Regression analysis of the reaction quantum yield as a function of the molar concentration of pyridine [PYR] up to a concentration of 0.2 M (at a fixed concentration of PNAP equal to 1.00×10^{-5} M) gave the relationship

$$\phi_E^a = 0.0169[\mathrm{PYR}] \quad , \tag{13}$$

with a correlation coefficient greater than 0.99. It should be noted that in each experiment, PNAP exhibited good first-order kinetics to 75% transformation. At pyridine concentrations greater than 0.2 M [Winterle (1984)], nonlinearity was observed. Hence, equation 13 is only valid to a 0.2 M concentration of pyridine. Further laboratory experiments were carried out at 366 nm and 25°C. The data indicated that ϕ_E^a was essentially the same, within experimental error, at 313 nm and 366 nm.

To determine the temperature dependence of the PNAP/PYR actinometer, photolysis rates were measured at 43°C, and then compared to the rates at 25°C. The rate of photolysis slowed down by 12% at 43°C. Using the standard Arrhenius equation, the energy of activation was found to be -1.3 kcalories. Therefore, in the range 1°C to 45°C, the photolysis rate constant of the PNAP/PYR actinometer will be within ± 25% of its value at 25°C. Thus, the rate constant for this actinometer, and hence its reaction quantum yield, is not very sensitive to changes in temperature.

The ultraviolet-visible absorption spectrum of PNAP was obtained in an aqueous solution containing 1% acetonitrile [Mill et al. (1982b)]. The molar absorptivities were calculated using the Beer-Lambert law as a function of wavelength (i.e. at the wavelength centers corresponding to the interval ranges listed in Table 1 of the Test Guideline) and the results are summarized in Table 1. PNAP shows absorption of light in the solar region $\lambda > 290$ nm to 410 nm with strong absorption in the region around 300 nm. As discussed in Section II.B.3.b.i., this actinometer is extremely useful since a large number of chemicals absorb light in this region.

An important variable for the PNAP/PYR actinometer is the absorption rate constant k_a^a, in days^{-1}, and is defined by the equation

TABLE 1. MOLAR ABSORPTIVITIES OF PNAP

λ center (nm)	$\varepsilon_{\lambda}^{a}(M^{-1}cm^{-1})$*
297.5	3790
300.0	3380
302.5	3070
305.0	2820
307.5	2590
310.0	2380
312.5	2180
315.0	1980
317.5	1790
320.0	1610
323.1	1380
330.0	959
340.0	561
350.0	357
360.0	230
370.0	140
380.0	81
390.0	45
400.0	23
410.0	0

---

*$\varepsilon_{\lambda}^{a}$ corresponds to the molar absorptivity at λcenter averaged over the interval range listed in Table 1 of the Test Guideline CG-6000.

119

$$k_a^a = \sum \varepsilon_\lambda^a L_\lambda \quad , \qquad (14)$$

where ε_λ^a is the molar absorptivity of PNAP and L_λ is the solar irradiance in water. The molar absorptivities of PNAP are listed in Table 1. Using this data with the L_λ values listed in Tables 3-6 of the Test Guideline (CG-6000), k_a^a has been calculated as a function of latitude and season of the year in the northern hemisphere. The results are summarized in Table 2 of the Test Guideline. This parameter is essential to carrying out the phase two aqueous photolysis experiments.

iii. Geometry of the Reaction Vessel

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this Test Guideline measures rate constants (k_p) in tubes (e.g., 13 x 100 mm). Such a reaction vessel receives radiation from all directions, due to scattering of the light off the walls, in contrast to a water body (e.g., a lake) which receives sunlight only from above. It is expected, therefore, that photoreactions would be faster in tubes. This was verified experimentally [Mill et al. (1982b)]. Laboratory experiments were carried out in tubes and dishes using the actinometers p-nitroacetophenone/pyridine, p-nitroanisole/pyridine, and rose bengal. The experiments using dishes represented a water body. All the data indicated that the ratio of rate constants of tubes to dishes was 2.2 ± 0.3. Thus,

$$k_p = \gamma\, k_{pE} \quad , \qquad (15)$$

with $\gamma = 2.2 \pm 0.3$. The factor γ is called the geometry factor. For tubes, therefore, $\gamma = 2.2$ while for water bodies $\gamma = 1.0$.

iv. Phase 2 Test Method

In phase two, a standard PNAP/PYR actinometer is used to measure sunlight intensities incident on the sample during photolysis. The rate constant for this actinometer, k_p^a, can be adjusted to match the approximate rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the reaction quantum yield of the actinometer, the rate constant can be adjusted according to equation 13.

Using equations 5 and 15, the rate constant in tubes is given by

$$k_p = \gamma \phi_E \sum \varepsilon_\lambda L_\lambda \quad . \qquad (16)$$

Therefore, the rate constants for the actinometer, k_p^a, and the test chemical, k_p^c, in tubes are

$$k_p^c = \gamma \phi_E^c \sum \varepsilon_\lambda^c L_\lambda \qquad (17)$$

$$k_p^a = \gamma\phi_E^a \sum \varepsilon_\lambda^a L_\lambda . \qquad (18)$$

Since the phase two test method involves the simultaneous photolysis of test chemical and actinometer in identical tubes, equation 18 can be divided into equation 17. Carrying out this operation and solving for ϕ_E^c yields

$$\phi_E^c = \frac{k_p^c}{k_p^a} \frac{\sum \varepsilon_\lambda^a L_\lambda}{\sum \varepsilon_\lambda^c L_\lambda} \phi_E^a \quad . \qquad (19)$$

The reaction quantum yield of the test chemical, ϕ_E^c, can be determined in the following way.

(1) Determination of the ratio (k_p^c/k_p^a) .

Using equation 6, the equations for the test chemical and actinometer in tubes are

$$\ln(C_o/C_t)^c = k_p^c t \qquad (20)$$

$$\ln(C_o/C_t)^a = k_p^a t \quad . \qquad (21)$$

Since the phase two test method involves the simultaneous photolysis of the test chemical and actinometer in identical tubes, equation 20 can be divided by equation 21. Carrying out this operation and rearranging yields

$$\ln(C_o/C_t)^c = (k_p^c/k_p^a)\ \ln(C_o/C_t)^a \quad . \tag{22}$$

$\ln(C_o/C_t)^c$ is a linear function of $\ln(C_o/C_t)^a$ with a slope given by

$$\text{slope} = (k_p^c/k_p^a) \quad . \tag{23}$$

The most precise and easiest method of determining the slope is by linear regression analysis on the experimental data. Therefore, by measuring the concentration of test chemical and actinometer at the same time as a function of time, these data can be fitted to equation 22 by linear regression analysis with $\ln(C_o/C_t)^a$ as the independent variable and $\ln(C_o/C_t)^c$ as the dependent variable to give the best straight line. The slope, the ratio of the rate constants, is given by equation 23.

(2) The term $\sum\varepsilon_\lambda^a L_\lambda$ for the actinometer has been tabulated as a function of latitude and season of the year [Table 2 in the Test Guideline].

(3) The term $\sum\varepsilon_\lambda^c L_\lambda$ for the Test Guideline can be obtained from the experimentally measured molar absorptivities (Section II.B.2) and the values of L_λ [listed in Tables 3 to 6 in the Test Guideline as a function of latitude and season of the year].

(4) ϕ_E^a can be determined from equation 13 at the molar concentration of pyridine used in the standard actinometer.

With the value of ϕ_E^c, ϵ_λ^c, and the appropriate L_λ values, k_{pE} for the test chemical can be calculated as a function of latitude and season of the year using equation 5. The corresponding half-life can be calculated using k_{pE} in equation 4. These rate constants and half-lives correspond to sunlight photolysis in water bodies under clear sky conditions and at shallow depths.

Photolysis experiments were carried out in the laboratory using a photochemical "merry-go-round" reactor (PMGRR) at 313 nm and in sunlight using the test chemicals dibenzothiophene, nitrobenzene, and p-methoxyacetophenone [Mill et al. (1982b)]. These data were used to calculate the laboratory reaction quantum yield at 313 nm [ϕ(313 nm)], the sunlight reaction quantum yield (ϕ_E^c), the half-life calculated from the laboratory data [$t_{1/2E}$(calc.)], and the half-life in sunlight [$t_{1/2E}$(obs.)]. These data are summarized in Table 2. Comparison of the corresponding quantum yield data and half-life data for the laboratory experiments with those in sunlight indicated that the results are in good agreement confirming the phase two test method.

TABLE 2. CALCULATED AND OBSERVED QUANTUM YIELDS AND ENVIRONMENTAL PHOTOLYSIS HALF-LIVES FOR SOME TEST CHEMICALS

Chemical	ϕ(313nm)	ϕ_E^c	$t_{1/2E}$(calc.) days	$t_{1/2E}$(obs.) days
Dibenzothiophene	5.0×10^{-4}	7.3×10^{-4}	9.1	6.4
Nitrobenzene	1.8×10^{-4}	1.9×10^{-4}	19.8	19.3
P-Methoxyaceto-phenone	9.5×10^{-4}	13×10^{-4}	11.9	12.0

4. Summary

This screening test has been designed to determine the molar absorptivity of a test chemical, ε_λ, and its reaction quantum yield, ϕ_E^c. These parameters can be used to determine environmentally relevant rate constants at low absorbance and shallow depths in pure water as a function of latitude and season of the year. Tables of solar irradiance (Tables 3-6) have been included in this Test Guideline to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp and Cline (1977) have written a computer program to calculate the rates of photolysis as a function of depth in water, in natural waters as a function of the attenuation coefficient of the water (α_λ), the average ozone layer thickness that pertains to the season and location of interest, and as a function of latitude and season of the year. This program has been recently updated with the best available solar irradiance data and is called the GC SOLAR computer program. The GC SOLAR computer program is available on request. [R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.]

This Test Guideline represents a screening test to obtain the reaction quantum yield of a chemical. Since it does not require sophisticated (and expensive) photochemical equipment, the reaction quantum yield can be obtained easily and at a modest cost. However, the reaction quantum yield can be obtained more precisely in the laboratory using special photochemical equipment and monochromatic light by procedures described by Zepp (1978) and Mill et al. (1982a). A reaction quantum yield obtained by one of these procedures is acceptable as a replacement for the determination of the reaction quantum yield in sunlight. An upper-tier test has been published as a Test Guideline in NTIS (CG-6010) which gives detailed procedures for determining the reaction quantum yield in the laboratory using a monochromatic light source.

C. Applicability and Specificity

This test method is applicable to all chemicals which have uv-visible absorptions in the range 290-800 nm. Solar radiation reaching the earth's surface has a sharp cutoff at a wavelength of approximately 290 nm due to the absorption by ozone [Leighton

(1961), Peterson (1976), Zepp and Cline (1977), Demerjian (1980)]. The long wavelength limit is set by thermochemistry since light of wavelength greater than 800 nm is not of sufficient energy to break chemical bonds of ground state molecules [Calvert and Pitts (1966), Benson (1976)]. Photolysis does not occur unless there is absorption of radiant energy. This is the direct consequence of the Grotthus-Draper law, the first law of photochemistry. If a chemical in aqueous solution only absorbs light at wavelengths below 290 nm it will not undergo direct photolysis in sunlight. A few examples of chemicals that only absorb light below 290 nm and therefore should not be tested by this Test Guideline are alkanes, alkenes, alkynes, saturated alcohols, and saturated acids. It is possible that some chemicals will absorb radiation mainly at wavelengths below 290 nm but may have an absorption tail that extends above 290 nm. Photolysis experiments should be carried out for these chemicals.

This Test Guideline is only applicable to pure chemicals and not to the technical grade. Overestimates of molar absorptivities usually occur when technical substances are tested because the impurities frequently absorb in the same spectral region as the pure chemical.

D. Rationale for the Selection of the Test Conditions

1. Special Laboratory Equipment

All the laboratory equipment needed to determine the uv-visible absorption spectrum of a test chemical in aqueous solution is listed in the references in Test Guideline CG-1050. The equipment required to carry out this test is standard equipment which is commonly used in most laboratories.

In order to carry out the photolysis experiments, special tubes are necesary to contain the reaction solutions during photolysis. Reaction tubes with 11 mm inside diameter are recommended as they are inexpensive, easy to seal to prevent volatilization of the chemical, and can be easily mounted on a rack in sunlight. Disposable culture tubes (13 x 100 mm) with Teflon-line screw caps are readily available from commercial laboratory supply companies. For some chemicals, it may be difficult to determine their concentrations in the small volumes present in 11 mm i.d. tubes. For such chemicals, the use of large reaction vessels is permissible as long as the pathlength is less than 0.5 meters. This is necessary in order that first-order kinetics are obeyed (Section II.B.1). Furthermore, the cell walls should be relatively thin to minimize the loss of sunlight through the cell walls. Reaction tubes of either quartz or thin-walled borosilicate glass may be used. If a chemical absorbs strongly at $\lambda < 340$ nm, then quartz tubes should be used. Quartz will transmit 100% of the sunlight in the region

290-340 nm while borosilicate glass will absorb some of this light. The absorption spectrum of the chemical in aqueous solution, as measured by Test Guideline CG-1050, can be used to determine which type of glass to use.

It is extremely important that all reaction tubes be capable of being sealed (without the use of grease) and be filled as completely as possible to prevent volatilization of test chemical or water (see Section II.C.6). Screw caps lined with Teflon inserts are recommended. Grease should be avoided since hydrophobic chemicals might adsorb it. Volatile compounds can be conveniently studied in culture tubes equipped with gas-tight Mininert® valves. Samples can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate [Zepp [1984)]. As an alternative, the tubes can be sealed with a torch.

2. Purity of Water

Pure water is used because dissolved impurities could sensitize or otherwise affect the rate of photolysis. In addition, the water needs to be sterile because bacteria may consume or alter the chemical substance during the prolonged periods of testing which may occur in the course of a rate determination. Thus, pure water [e.g., ASTM Type II A (ASTM 1979)], or an equivalent grade, is recommended in this Test Guideline. Furthermore, it is important that the water be saturated with air prior to preparation of the test and control solutions to simulate environmental conditions. Air-saturated water can be easily prepared by allowing the water to equilibrate in a vessel plugged with sterile cotton.

3. Sterilization

Sterilization is necessary to kill the bacteria and therefore eliminate or minimize biodegradation which could interfere with the photolysis rate determination. The presence of bacteria in either the test solutions or controls may cause biodegradation of the test substance, especially when the photolysis experiments are carried out over a long period of time. This will introduce an error in the concentration of the test chemical. Thus, it is extremely important to use aseptic conditions in carrying out all photolysis experiments to minimize biodegradation. Glassware can be sterilized easily in an autoclave or by use of any other suitable non-chemical method.

4. Concentration of Solution

It is extremely important that solutions of chemical substances used in this Test Guideline be prepared at low concentrations in order to approximate environmental conditions and to allow first-order kinetics assumptions to apply (see Section II.B.I.). Furthermore, an absorbance of 0.05 has been adopted in this Test Guideline since solutions with this absorbance can be easily achieved in the laboratory. This absorbance limit yields an 11% error, an acceptable error for a screening test (Section II.B.1).

If the chemical substance is too difficult to dissolve in pure form to permit reasonable handling and analytical procedures, then a test solution may be prepared by first dissolving a chemical in reagent grade acetonitrile. The final acetonitrile concentration in the test solution should be no more than one volume percent in order to avoid solvent effects (Smith et al. 1977, 1978). Acetonitrile was chosen as a solvent as it is soluble in water, is non-polar and thus effective in dissolving many substances which are insoluble in water, it does not absorb radiation over the wavelength range of 290 to 800 nm, and it causes minimal solvent effects (i.e., minimum shifts in bands and changes in absorbance) for test substances.

5. pH Effects

The molecular structure of a chemical substance which ionizes or protonates is a function of the pH. As a result, the absorption spectrum and consequently the rate of photolysis may change with pH. The recommended procedure is to determine the uv-visible spectra under conditions in which only one species strongly predominates in solution. This can be accomplished by preparing buffered solutions containing the test chemical at pHs at least 2 units above the pK_a and at least 2 units below the

pK_a. For example, the pK_a for phenol is 9.89. At a pH below 7.89, the protonated form of the phenol strongly predominates; at a pH of greater than 11.89, the phenoxide form of the phenol strongly predominates. The reaction quantum yield for ionizable compounds should also be determined in buffered solutions, where only one species strongly predominates. Thus, photolysis experiments should be carried out at the same pHs used to obtain the spectrum of each species.

Since buffers could influence the rate of photolysis, the recommended buffers for use in this Test Guideline were carefully chosen to be transparent to radiation between 290 and 800 nm and should be kept at very low concentrations to avoid buffer effects which may cause transformation of the substance by, for example, catalysis.

6. Control Solution

Undetected loss of a test substance through volatilization, hydrolysis, or other processes during the course of the photolysis experiment will result in the determination of erroneously large rate constants for aqueous photolysis. Therefore, for volatile chemical substances, it is important that the reaction vessels and control vessels be filled as completely as possible and sealed in order to avoid evaporative losses. To correct for possible losses, control solutions of test substance, in darkened vessels, are placed side-by-side with the photolysis vessels and the contents of the control vessels are analyzed at prescribed times during the experiment and at the end of the experiment. In this way, the loss of test chemical for processes other than photolysis may be detemined and eliminated or accounted for in determining k_p. For simplicity, if the loss of chemical in the control is small (i.e., approximately 10% or less), one can calculate a first-order loss, k_{loss}, and subtract it from $(k_p)_{obs}$. to give the corrected direct photolysis rate constant k_p. If hydrolysis is found to be significant (i.e., greater than 10%), hydrolysis studies should be carried out first to define precisely the kinetics of this process [Test Guideline CG-5000].

7. Absorption Spectrum as a Criterion for Performing the Aqueous Photolysis Test

The test method is applicable to all chemicals that have uv-visible absorptions in the range 290-800 nm (section II.C). Thus, the uv-visible absorption spectrum of a chemical in aqueous solution will give a good indication of whether it would be useful to carry out sunlight aqueous photolysis tests.

8. Sunlight Actinometer

In order to quantify the rate of photolysis more precisely, it is necessary to measure the sunlight intensity incident on the sample during photolysis. A standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) has been developed and the details have been discussed in Section II.B.3.b.ii. According to equation 5, the rate constant is a function of the reaction quantum yield. Furthermore, the reaction quantum yield can be adjusted by varying the molar concentration of pyridine according to equation 13. Hence, by varying the pyridine concentration, the PNAP photolysis rate constant can be adjusted so that the half-life can range from several hours to several weeks. In order to make the actinometer operable, it is necessary that [PYR] > 10 [PNAP] and < 0.2 M. Therefore, the concentration of PNAP must be approximately 1×10^{-5} M. In the recommended actinometer formulation, the concentration of PNAP is set at 1.00×10^{-5} M. The concentration required to adjust the rate of the actinometer to equal the rate of the test chemical can be derived as follows. Since it is necessary to adjust the rate of photolysis of the chemical to that of the test chemical, then

$$k_p^c = k_p^a \quad . \tag{23}$$

According to equation 15, the rate constant for the actinometer in tubes is

$$k_p^a = 2.2k_{pE}^a \quad . \tag{24}$$

However, k_{pE}^a for the actinometer can be obtained from equation 5,

$$k_{pE}^{a} = \phi_{E}^{a}\sum\varepsilon_{\lambda}^{a}L_{\lambda} \quad , \qquad (5)$$

and since k_{a}^{a} for the actinometer is $\sum\varepsilon_{\lambda}^{a}L_{\lambda}$,

$$k_{pE}^{a} = \phi_{E}^{a}\ k_{a}^{a} \quad . \qquad (25)$$

Substitution of equation 25 into equation 24 yields

$$k_{p}^{a} = 2.2\phi_{E}^{a}\ k_{a}^{a} \quad . \qquad (26)$$

According to equation 13, $\phi_{E}^{a} = 0.0169[PYR]$. Substituting this result in equation 26 yields

$$k_{p}^{a} = 0.0372[PYR]\ k_{a}^{a} \quad . \qquad (27)$$

Finally, substituting equation 27 into equation 23 and solving for [PYR] yields

$$[PYR] = 26.9\ (k_{p}^{c}/k_{a}^{a}) \ . \qquad (28)$$

The term k_{p}^{c} is the sunlight rate constant for the test chemical in tubes and the variable $k_{a}(=\sum\varepsilon_{\lambda}^{a}L_{\lambda})$ for the actinometer can be obtained from Table 2 of the Test Guideline.

For 1 liter of solution, the mass of pyridine required to adjust the rate of the actinometer is

$$\text{mass(grams)} = \text{F.W. [PYR]} \quad , \tag{29}$$

where F.W. = 79.10 g/mole, the formula weight of pyridine. The density of pyridine is 0.9819 g/mL at 20°C [Weast (1973-1974)]. Thus, the volume of pyridine at 20°C required to equal this mass is

$$\text{V(mL)} = 80.6 \text{ [PYR]} \quad . \tag{30}$$

9. Solar Irradiance Data

In order to calculate the reaction quantum yield of the test chemical, ϕ^c_E, and then calculate k^c_{pE} and $t^c_{1/2E}$, it is necessary to use solar irradiance data L_λ. The theoretical basis for the L_λ data is discussed in the following paragraphs.

In a water body exposed to the sun, the intensity of light passing into a water body is composed of light directly from the sun ($I_{d\lambda}$) and the sunlight from the sky ($I_{s\lambda}$). Leighton (1961) discussed the calculation and contribution of direct and sky light to the total intensity of sunlight filtering down through the troposphere at a point on the earth's surface as a function of the zenith angle Z, Figure 1. Zepp and Cline (1977) extended the treatment to the behavior of $I_{d\lambda}$ and $I_{s\lambda}$ that enter a water body, Figure 1. Part of the theoretical treatment has been summarized in Section II.B.1 and the following discussion extends this treatment. According to equation 2

$$k_{pE} = \phi_E k_a = \phi_E \sum k_{a\lambda} \quad . \qquad (2)$$

Furthermore, Zepp and Cline showed that

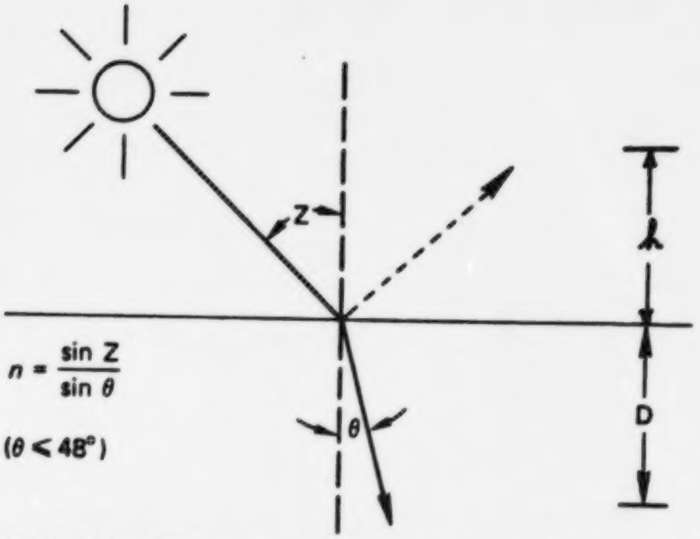
$$k_{a\lambda} = 2.303\ \epsilon_\lambda j^{-1} Z_\lambda \quad , \qquad (31)$$

where

$$Z_\lambda = I_{d\lambda} \sec\theta + 1.2\ I_{s\lambda} \quad . \qquad (32)$$

In equation 31, the term ϵ_λ is the molar absorptivity of a chemical in aqueous solution, j is a constant which converts the intensity units into units that are compatible with the concentration units of C for the chemical (i.e., $j = 6.02 \times 10^{20}$ when C is the molar concentration of the chemical). In equation 32, $I_{d\lambda}$ and $I_{s\lambda}$ are the direct and sky sunlight intensity in photons cm^{-2} $sec.^{-1}$, and θ is defined by the refractive index of water, Figure 1. Z_λ is the actinic irradiance in water at shallow depths in the units of photons cm^{-2} $sec.^{-1}$. Zepp and Cline (1977) tabulated Z_λ values for 40°N latitude as a function of midseason dates for spring, summer, winter, and fall. Substitution of equation 31 into equation 2 yields

$$k_{pE} = 2.303 j^{-1} \theta_E \sum \epsilon_\lambda Z_\lambda \quad . \qquad (33)$$


Z
λ
D
θ
$n = \frac{\sin Z}{\sin \theta}$
($\theta < 48°$)

Z = Angle of incidence (solar zenith angle in case of direct sunlight)
θ = Angle of refraction
n = Refractive index of water

FIGURE 1 REFLECTION AND REFRACTION OF A LIGHT BEAM PASSING FROM ATMOSPHERE INTO A WATER BODY
(From Zepp and Cline, 1977)

Based on their theoretical work, Zepp and Cline have written a computer program for 39 specific wavelength intervals from 296.3 to 825nm which calculates instantaneous rate constants at specific times of day for a given latitude and midseason date and summed them up to obtain k_{pE}. This program has been recently updated with the best available solar irradiance date and is called the GG SOLAR computer program [Zepp (1984)]. The term midseason date used by Zepp and Cline does not refer to a day midway in the season, but rather to a day during the season when the angle of declination of the sun is at specific values (listed in a subsequent paragraph). The program further calculates a photolysis rate constant k_{pE}, which is averaged over a 24-hour calendar day in the units of days^{-1}. Required data inputs are: (1) molar absorptivities of the chemical at wavelengths greater than 297.5 nm; (2) the refractive index of the medium (e.g., for water n = 1.34); (3) the attenuation coefficient, α_λ, of the medium (e.g., α_λ for distilled water or for natural water bodies); (4) the reaction quantum yield, ϕ_E^c, of the chemical; (5) the solar declination, solar right ascension, and siderial time for the date of interest obtained from the Astronomical Almanac [AA (1982)]; (6) the latitude and longitude; and (7) the average ozone layer thickness that pertains to the season and location of interest.

For routine computations, the program calculates rate constants k_{pE} and half-lives for midseason dates corresponding to solar declinations of +10° for spring, +20° for summer, -10°

for fall, and -20° for winter, as a function of local time of day, longitude, and latitude for 0° to 90°N in 10° increments.

To obtain the L_λ data, Mill et al. (1982a), used the Zepp and Cline computer program with $\epsilon_\lambda = 1$, $\phi = 1$, and the absorption coefficients α_λ for pure water. The L_λ values were obtained for the midseasons of winter, spring, summer, and fall for the latitudes 20° to 50°N and these data are listed in Tables 3 to 6 of the Test Guideline. The L_λ values correspond to the 1982 midseason dates of January 21, April 16, July 24, and October 20 [AA (1982)]. The values of L_λ are in the units of 10^{-3} einsteins cm^{-2} day^{-1}. The L_λ values are applicable to clear sky conditions, water bodies, shallow depths, and for chemicals with very weak absorbance in pure water. The Zepp and Cline computer program has been recently updated and is called the GC SOLAR program [Zepp (1984)]. Mill et al. (1984) used this program with $\epsilon_\lambda = 1$, $\phi = 1$, and the absorption coefficient α_λ for pure water to obtain updated L_λ values as a function of latitude and season of the year. These L_λ values are summarized in Tables 3-6 of the Test Guideline.

The solar irradiance Z_λ data pertain to midday, midseason, values in the units of photons cm^{-2} sec^{-1} while the solar irradiance L_λ data represent day-averaged, midseason, values in the units of 10^{-3} einsteins cm^{-2} day^{-1}. The L_λ data are used in equation 5 to calculate day-averaged, midseason, direct photolysis rate constants. The Z_λ data are used in equation 33 to calculate midday, midseason, direct photolysis rate constants.

10. Geometry of the Reaction Vessel

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this Test Guideline measures rate constants in tubes (e.g., 13 x 100 mm). Such a reaction vessel receives radiation from all directions, due to scattering of the light off the walls, in contrast to a water body such as a lake which receives sunlight only from above. It is expected, therefore, that photoreactions would be faster in tubes. This was verified experimentally [Mill et al. (1982b)] and this has been discussed in detail in Sections II.B.3.b.iii. For these experiments, tubes are the simplest and easiest reaction vessels to use since they can be sealed easily to minimize or eliminate volatilization and can be handled easily. Hence, this Test Guideline recommends the use of tubes as reaction vessels and the method has been modified to take into account the increased rate in tubes (equation 15).

11. Chemical Analysis of Solutions

The analytical techniques employed in the determination of the concentration of the test substances are left to selection by the tester. This is in recognition of the many different techniques available and the practical advantage of being able to make particular use of one of the properties of the substance; e.g., the NMR or UV spectrum of the substance, or its chromatographic behavior. Analytical techniques that permit the determination of the test compound to the exclusion of impurities or photolysis reaction products are recommended to the extent practicable. Therefore, chromatographic techniques are particularly desirable. Whenever practicable, an analytical procedure should be used which has a precision of ±5% or better. The specific technique which is utilized should be adequately described.

The p-nitroacetophenone in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 2.5% acetic acid, 50% acetonitrile, and 47.5% water which is passed through the column at a flow rate of 2 mL/minute. This analytical procedure was specifically developed to analyze for PNAP, especially at high pyridine concentrations (i.e., for pyridine concentrations close to 0.2 M) [Mill et al. (1982b), Winterle (1984)].

12. Outdoor Experimental Conditions

It is important that the photolysis reaction vessels containing the chemical substance be placed in an area free from shade and reflections and on a black, non-reflecting, background to ensure that they only receive direct and sky radiation from the sun. The reaction vessels should be tilted at 30° from horizontal with the upper end pointing due north in the northern hemisphere so that they present a large surface area and minimum pathlength to the sun and create minimal internal reflections.

It is recommended that the photolysis experiments be carried out during a frost-free time of the year (i.e., May, June, July, August, or September in the northern hemisphere--temperature permitting). This period of time was chosen because the solar intensity is a maximum and consequently the rate of photolysis will be a maximum. Thus, the kinetics of photolysis will be easier to follow, especially for chemicals that photolyze slowly. Furthermore, in many parts of the United States, the temperature falls below 0°C during the winter months. Therefore, if the photolysis experiments are carried out in the winter, the dilute aqueous reaction solution would freeze, the tubes would break, and the samples would be destroyed. It may be possible to avoid this problem by placing the reaction tubes in a housing transparent to the appropriate wavelengths of radiation and thermostated to control the temperature at 25 ±5°C. Thus, with this device it may be possible to carry out photolysis experiments at any time of the year. However, transmission of sunlight through the housing could present a difficult problem to solve.

III. REFERENCES

AA. 1982. Astronomical Almanac.

ASTM 1978. Annual book of ASTM standards, American Society for Testing and Materials, Philadelphia, PA., Part 31, Method D.

Benson WR, Lombardo P, Egry IJ, Ross, RD, Barron RP, Mastbrook DW, Hansen EA. 1971. Chlordane photoalteration products: Their preparation and identification. J Agr Food Chem 19:857-862.

Benson SW. 1976. Thermochemical kinetics. Second edition. Wiley-Interscience, N.W., N.Y.

Broderius SJ and Smith LC. 1980. Direct photolysis of hexacyanoferrate complexes. EPA-600/3-80-003.

Calvert JG and Pitts JN, Jr. 1966. Photochemistry. Wiley, N.Y., N.Y.

Carey JH and Fox ME. 1981. Photodegradation of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). 1. Pathway of the direct photolysis in solution. J. Great Lakes Res. 7:234.

Crossland NO and Wolff CJM. 1984. The fate and biological effects of pentachlorophenol in outdoor ponds. I. Fate: Predicted and observed. Submitted for publication to Aquatic Tox.

Demerjian KL, Schere KL, and Peterson JT. 1980. Theoretical estimates of actinic (spherically integrated) flux and photolysis rate constants of atmospheric species in the lower troposphere. Adv Sci and Tech 10:389.

Dulin D and Mill T. 1982. Development and application of solar actinometers. Environ Sci and Technol 16:815.

Grunwell JR, Erickson RH. 1973. Photolysis of parathion. New products. J Agr Food Chem 21:929-931.

Langford CH, Wingham M, Sastrin VS. 1973. Ligand photooxidation in copper (II) complexes of nitrilotriacetic acid. Environ Sci and Technol 7:820-822.

Leighton PA. 1961. Photochemistry of air pollution. New York: Academic Press, Inc.

Mabey WR, Tse D, Baraze A and Mill T. 1983. Photolysis of nitroaromatics in aquatic systems. I. 2,4,6-trinitrotoluene. Chemosphere 12:3.

Mancini JL. 1978. Analyis framework for photodecomposition in water. Environ Sci and Technol 12:1274-1276.

Mill T, Davenport JE, Dulin DE, Mabey WR and Bawol R. 1981. Evaluation and optimization of photolysis screening protocols. U.S. Environmental Protection Agency. EPA 560/5-81-003.

Mill T, Mabey WR, Bomberger DC, Chou T-W, Hendry DG, and Smith JH. 1982a. Laboratory protocols for evaluating the fate of organic chemicals in air and water. EPA-600/3-82-022.

Mill T, Mabey WR, Hendry DG, Winterle J, Davenport J, Barich V, Dulin D, and Tse D. 1982b. Design and validation of screening and detailed methods for environmental processes. EPA-

Mill T, Davenport JE, Winterle JS, Mabey WR, Drossman H, Tse D, and Liu A. 1984. Toxic substances process data generation and protocol development. EPA Contract No. 68-03-2981 with EPA Athens Research Laboratory, Office of Research and Development. Draft final report.

Peterson JT. 1976. Calculated actinic fluxes (290-700nm) for air pollution photochemistry applications.

Prutton CF and Maron SH. 1951. Fundamental principles of physical chemistry. Chapter XXll. The Macmillan Co. New York, New York.

Simmons MS and Zepp RG. 1984. The influence of humic substances on photolysis of nitroaromatics in aqueous systems. Submitted for publication to Water Res.

Smith JH, Mabey WR, Bohonos N, Holt BR, Lee SS, Chou T-W, Bomberger DC, Mill T. 1977. Environmental pathways of selected chemicals in freshwater systems. Part I. Background and experimental procedures. U.S. Environmental Protection Agency. EPA 600/7-77-113.

Smith JH, Mabey WR, Bohonos N, Holt BR, Lee SS, Chou T-W, Bomberger DC, Mill T. 1977. Environmental pathways of selected chemicals in freshwater systems. Part II. Laboratory studies. Athens, GA: U.S. Environmental Protection Agency. EPA 600/7-78-074.

Su CC and Zabik MJ. 1972. Photochemistry of bioactive compounds. Photolysis of arylamidine derivatives in water. J Agr Food Chem 20:320-323.

USEPA. 1979. U.S. Environmental Protection Agency. Office of Toxic Substances. Toxic Substances Control: Discussion of premanufacture testing policy and technical issues. Request for comment. Federal Register 44, 16240.

Winterle J. 1984. Private communication.

Weast RC. 1973-1974. Handbook of chemistry and physics. The Chemical Rubber Company, Cleveland, Ohio.

Wolfe, NL, Zepp RG, Schlotzhauer PF, and Sink M. 1982. Transformation pathways of hexachlorocyclopentadiene in the aquatic environment. Chemosphere 11:91.

Wolfe, NL, Zepp RG, Baughman GL, Fincher RC, Gordon JA. 1976. Chemical and photochemical transformation of selected pesticides in aquatic systems. Athens, GA: U.S. Environmental Protection Agency. EPA 600/3-767-067.

Zepp RG, Wolfe NL, Gordon JA, Baughman GL. 1975. Dynamics of 2,4-D esters in surface water. Hydrolysis, photolysis, and vaporization. Environ Sci and Technol 9:1144-1150.

Zepp RG, Wolfe NL, Gordon JA, Fincher RC. 1976. Light-induced transformations of methoxychlor in aquatic systems. J Agr Food Chem 24:727-733.

Zepp RG, Wolfe NL, Azarraga LV, Cox RH, Pape LW. 1977. Photochemical transformation of the DDT and methoxychlor degradation products, DDE and DMDE, by sunlight. Arch Environ Contam Toxicol 6:305-314.

Zepp RG and Cline DM. 1977. Rates of direct photolysis in aquatic environment. Environ Sci Technol 11:359-366.

Zepp RG. 1978. Quantum yields for reaction of pollutants in dilute solution. Environ Sci and Technol 12:327.

Zepp RG. 1982. Experimental approaches to environmental photochemistry. The handbook of environmental chemistry. O. Hutzinger, Editor. Springer-Verlag.

Zepp RG. 1983. Mathematical modeling of DDE in a quarry. Kinetics of photolysis of DDE. Unpublished results, U.S. EPA, Athens, GA.

Zepp RG. 1984. Private communication.

Zepp RG. 1982a. Photochemical transformations induced by solar ultraviolet radiation in marine ecosystems. In: The role of solar ultraviolet radiation in marine ecosystems. Calkins J, Editor. Plenum Publishing Corporation, N.Y.

IV. APPENDIX: GLOSSARY OF IMPORTANT SYMBOLS

PYR	Pyridine
PNAP	p-Nitroacetophenone
λ	Wavelength λ
A_λ	Absorbance at wavelength λ.
a	Actinometer (composed of PNAP/PYR).
ε_λ^c	Molar absorptivity of a chemical C.
ε_λ^a	Molar absorptivity of the actinometer.
ℓ	light pathlength; the distance traveled by a beam of light passing through the system.
ϕ_E^c	Sunlight reaction quantum yield of chemical c in water.
ϕ_E^a	Sunlight reaction quantum yield of the actinometer in water. Since the reaction quantum yield is independent of λ, $\phi_E^a = \phi^a$ (i.e., the reaction quantum yield of the actinometer measured in the laboratory).
[C]	Molar concentration of chemical c.
[PYR]	Molar concentration of pyridine.
$- d[C]/dt$	Direct photolysis rate of chemical c.
k_{pE}	Direct photolysis sunlight rate constant in water bodies in the environment.
$(k_{pE})_{max.}$	Maximum direct photolysis sunlight rate constant in water bodies in the environment.
k_p^c	Direct photolysis sunlight rate constant of chemical c in water in tubes.
k_p^a	Direct photolysis sunlight rate constant of the actinometer in water in tubes.
$k_{a\lambda}$	Specific light absorption of a photoreactive chemical at a low concentration and at wavelength λ.

k_a	Specific light absorption rate constant integrated over all wavelengths absorbed by the chemical.
k_a^a	Specific light absorption rate constant integrated over all wavelengths absorbed by the actinometer.
$t_{1/2}$	Sunlight half-life of a chemical in water in tubes.
$(t_{1/2E})_{min.}$	The minimum sunlight half-life of a chemical in water bodies in the environment.
I	The numbers of photons of light of wavelength λ in the system per cm^2 per second.
L_λ	Solar irradiance in water in the units 10^{-3} einsteins cm^{-2} day^{-1}.
γ	The geometry factor which represents the ratio of the rate constants in tubes (k_p) to the rate constant in water bodies in the environment (k_{pE}).

CG-6010
October, 1984

LABORATORY DETERMINATION OF THE DIRECT PHOTOLYSIS REACTION QUANTUM YIELD IN AQUEOUS SOLUTION AND SUNLIGHT PHOTOLYSIS

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

DISCLAIMER

Certain commercial equipment, instruments, and materials are identified in this Test Guideline in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the Environmental Protection Agency, nor does it imply that the material, instruments, or equipment identified are necessarily the best available for the purpose.

Contents

Page

I. INTRODUCTION 1
  A. Background and Purpose 1
  B. Definitions and Units 3
  C. Principle of the Test Method 6
  D. Applicability and Specificity 15

II. TEST PROCEDURES 18
  A. Test Conditions 18
    1. UV-Visible Absorption Spectrophotometer 18
    2. Special Photochemical Laboratory Equipment 18
      a. Design of the Apparatus 19
        i. Photochemical "Merry-Go-Round" Reactor 19
        ii. Photochemical Optical Bench 21
      b. Light Sources 22
      c. Light Filtering Systems 22
      d. Reaction Cells 23
    3. Cell Pathlength 25
    4. Solvents 25
    5. Sterilization 26
    6. pH Effects 26
    7. Volatile Chemical Substances 27
    8. Control Solution 27
    9. Absorption Spectrum as a Criterion for Performing the Reaction Quantum Yield Experiments 28
    10. Actinometers 28
      a. Low Optical Density Actinometers 29
        i. p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR) 29
        ii. p-Nitroanisole-Pyridine Actinometer (PNA/PYR) 31
      b. High Optical Density Actinometer (Ferrioxalate Actinometer) 33

Contents (con't)

Page

11. Chemical Analysis of Solutions 34
    - a. Chemical Analysis of Test Chemical Solutions 34
    - b. Chemical Analysis of p-Nitroacetophenone (PNAP) 34
    - c. Chemical Analysis of p-Nitroanisole (PNA) 34
    - d. Chemical Analysis of Ferrous Ion in the Ferrioxalate Actinometer 35

B. Procedure One: Determination of Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method 35

1. Phase 1 Experiments: UV-Visible Absorption Spectra 35
2. Phase 2 Experiments: Trial Photolysis Experiments 37
    - a. Determination of the Approximate Rate Constant of the Test Chemical.... 37
        - i. Preparation of Buffer Solutions 37
        - ii. Preparation of Test Chemical Solution 38
        - iii. Performance of the Test 38
    - b. Actinometry Experiments 40
        - i. Preparation of Actinometer Solutions 40
        - ii. Performance of the Test 42
3. Phase 3 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical 43

C. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method 44

1. Phase 1 Experiments: UV-Visible Absorption Spectra 44

Contents (con't)

Page

2. Phase 2 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical 45
    a. Preparation of Buffer Solutions 45
    b. Preparation of Test Chemical Solution 45
    c. Preparation of Ferrioxalate Actinometer Solution 45
    d. Determination of the Quantum Yield of the Test Chemical 46

III. DATA AND REPORTING........ 47

A. Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method 47

1. Phase 1 Experiments: UV-Visible Absorption Spectra 47
    a. Treatment of Results 47
    b. Test Data Report 49

2. Phase 2 Experiments: Trial Photolysis Experiments 51
    a. Determination of the Approximate Rate Constant of the Test Chemical 51
        i. Treatment of Results 51
        ii. Specific Analytical and Recovery Procedures 52
        iii. Other Test Conditions 52
        iv. Test Data Report 53
    b. Actinometry Experiments 54
        i. Treatment of Results 54
        ii. Test Data Report 54

3. Phase 3 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical 55
    a. Treatment of Results 55
    b. Other Test Conditions 57
    c. Test Data Report 58

Contents (con't)

Page

B. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method 60

  1. Phase 1 Experiments: UV-Visible Absorption Spectra 60
  2. Phase 2 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical 60

    a. Treatment of Results 60
    b. Specific Analytical and Recovery Procedures 62
    c. Other Test Conditions 62
    d. Test Data Report 63

IV. REFERENCES 65

V. APPENDIX 67

Hypothetical Illustrative Example: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method and Sunlight Photolysis 67

154

CG-6010 (October, 1984)

LABORATORY DETERMINATION OF THE DIRECT PHOTOLYSIS REACTION QUANTUM YIELD IN AQUEOUS SOLUTION AND SUNLIGHT PHOTOLYSIS

I. INTRODUCTION

A. Background and Purpose

Numerous chemicals enter natural aquatic water bodies from a variety of sources. Some pollutants present in aqueous media can undergo photochemical transformation in sunlight by direct photolysis. Therefore, quantitative data in the form of rate constants and half-lives are needed to determine the importance of direct photochemical transformation of pollutants in aqueous media.

Test Guideline CG-6000, the first in a series of aqueous photolysis test methods, was designed to determine the molar absorptivity and reaction quantum yield of a test chemical in aqueous solution. These parameters can be combined with solar irradiance data to determine environmentally relevant rate constants and half-lives in aqueous solution as a function of latitude and season of the year anywhere in the United States.

Test Guideline CG-6000 was developed as a screening test to obtain the direct photolysis reaction quantum yield of a chemical in aqueous solution by carrying out photolysis experiments in sunlight. This method does not require sophisticated and expensive photochemical equipment and therefore the reaction quantum yield can be easily determined and at a modest cost. However, there are circumstances when this method may not be applicable. For example, this procedure is not applicable for determining the reaction quantum yield of a test chemical when the temperature outdoors falls below zero degrees centrigrade. Furthermore, depending upon the status of a risk assessment for a specific chemical, a more precise value of the reaction quantum yield may be required. Thus, a more comprehensive procedure is needed to determine the direct photolysis reaction quantum yield in the laboratory using specialized photochemical equipment and monochromatic (or narrow band) light. This Test Guideline (CG-6010) describes laboratory procedures for determining the direct photolysis reaction quantum yield in aqueous solution.

The reaction quantum yield obtained in this Test Guideline can be combined with molar absorptivity data and solar irradiance data to determine environmentally relevant rate constants and half-lives in aqueous solution as a function of latitude and season of the year anywhere in the United States.

The procedures described in this Test Guideline are very detailed and the theory of photolysis in aqueous solution is relatively complicated. In order to follow these procedures, it is recommended that the Technical Support Document should be first studied carefully.

B. Definitions and Units

(1) "Radiant energy", or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.

(2) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_o) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ. Thus, $A_\lambda = \log_{10} (I_o/I)$.

(3) The "Beer-Lambert law" states that the absorbance of a solution of a given chemical species, at a fixed wavelength, is proportional to the thickness of the solution (ℓ), or the light pathlength, and the concentration of the absorbing species (C).

(4) "Molar absorptivity (ε_λ)" is defined as the proportionality constant in the Beer-Lambert law when the concentration is given in terms of moles per liter (i.e., molar concentration). Thus, $A_\lambda = \varepsilon_\lambda C\ell$, where A_λ and ε_λ represent the absorbance and molar absorptivity at wavelength λ and ℓ and C are defined in (3). The units of ε_λ are $\text{molar}^{-1}\ \text{cm}^{-1}$. Numerical values of molar absorptivity depend upon the nature of the absorbing species.

(5) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.

(6) A "zero-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is independent of the concentration of the chemical or the concentration of any other chemical present in the reaction mixture.

(7) The "first-order half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical to be reduced to one-half its initial value.

(8) The "sunlight direct aqueous photolysis rate constant (k_{pE})" is the first-order direct photolysis rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical dissolved in a water body in sunlight.

(9) The "solar irradiance in water (L_{λ}) " is related to the sunlight intensity in water at shallow depths and is proportional to the average light flux (in the units of 10^{-3} einsteins cm^{-2} day^{-1}) that is available to cause photoreaction in the wavelength interval centered at λ over a 24-hour day at a specific latitude and season date.

(10) "The Grotthus-Draper law", the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.

(11) The "Stark-Einstein law", the second law of photochemistry, states that only one molecule is activated to an excited state per photon or quantum of light absorbed.

(12) The "reaction quantum yield (ϕ_λ)" for an excited state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ. It is the ratio of the number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ.

(13) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

As a convenient reference, a glossary of the important symbols used in this Test Guideline is given in Section IV of the Support Document [CS-6010].

C. Principle of the Test Method

This Test Guideline is based on the principles developed by Zepp (1978), the use of low optical density actinometers developed by Mill et al. (1981, 1982) and Dulin and Mill (1982), and the high optical density ferrioxalate actinometer developed by Parker (1953) and Hatchard and Parker (1956).

Zepp (1978) published a paper on the determination of the reaction quantum yield for the reaction of pollutants in dilute aqueous solution in the laboratory. Based on this work, two procedures are described to determine the reaction quantum yield of a test chemical at low optical density in the laboratory. These procedures involve the use of: (1) low optical density test chemical and actinometer; and (2) low optical density test chemical and high optical density actinometer.

(1) Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method

For a low optical density test chemical and actinometer in which the absorbance of aqueous solutions is less than 0.02, the reaction quantum yield of a test chemical ϕ_c at wavelength λ is given by the equation

$$\phi_c = (k_{pc}/k_{pa})(\varepsilon_{\lambda a}/\varepsilon_{\lambda c})\,\phi_a \quad , \qquad (1)$$

where $\varepsilon_{\lambda a}$ and $\varepsilon_{\lambda c}$ are the molar absorptivities of the actinometer and test chemical, respectively, at wavelength λ, ϕ_a is the reaction quantum yield of the actinometer at wavelength λ, and k_{pa} and k_{pc} are the first-order direct photolysis rate constants for actinometer and test chemical, respectively. These rate constants are defined by the equations

$$\ln(C_o/C_t)_a = k_{pa}t \qquad (2)$$

$$\ln(C_o/C_t)_c = k_{pc}t \quad , \qquad (3)$$

where $(C_o)_a$ and $(C_t)_a$ are the molar concentrations of actinometer at time t = 0 and t and $(C_o)_c$ and $(C_t)_c$ are the molar concentrations of test chemical at time t = 0 and t. Since k_{pa} and k_{pc} are first-order rate constants, the half-lives of actinometer and test chemical are:

$$(t_{1/2})_a = 0.693/k_{pa} \qquad (4)$$

$$(t_{1/2})_c = 0.693/k_{pc} \quad . \qquad (5)$$

If both the actinometer and test chemical solutions are photolyzed in identical cells, equation 3 can be divided into equation 2. Carrying out this operation and rearranging the resultant equation yields

$$\ell n(C_o/C_t)_c = (k_{pc}/k_{pa})\ell n(C_o/C_t)_a \quad . \qquad (6)$$

Procedure One involves the simultaneous photolysis of test chemical and actinometer in an Ace-type photochemical "merry-go-round" reactor (PMGRR) using monochromatic light of wavelength λ . Two low optical density actinometers have been developed by SRI International for the Office of Toxic Substances/U.S. Environmental Protection Agency to measure the light intensity incident on the sample during photolysis [Mill et al. (1981, 1982) and Dulin and Mill (1982)]. These actinometers are: (1) p-nitroacetophenone-pyridine actinometer (PNAP/PYR); and (2) p-nitroanisole-pyridine actinometer (PNA/PYR). The rate constant of each of these actinometers can be adjusted to match the rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the reaction quantum yield of the actinometer, the rate constant can be adjusted according to the following equations:

PNAP/PYR actinometer $\phi_a = 0.0169[\mathrm{PYR}]$ (7)

PNA/PYR actinometer $\phi_a = 0.437[\mathrm{PYR}] + 0.000282$, (8)

where [PYR] is the molar concentration of pyridine for a PNAP or PNA concentration of approximately 1×10^{-5} M.

The laboratory procedure for determining the reaction quantum yield of a test chemical in aqueous solution has been divided into three phases using a uv-visible absorption spectrophotometer, an Ace-type PMGRR, and a 450 watt medium pressure mercury lamp with appropriate filters to isolate the monochromatic wavelength λ . In Phase 1, the molar absorptivities of a test chemical, ε_{313c} and ε_{366c} , are determined with a uv-visible absorption spectrophotometer using procedures outlined in Test Guidelines CG-1050 and CG-6000. Based on these results, photolysis experiments are carried out at 313 or 366 nm corresponding to the higher value of ε_{313c} or ε_{366c} .

The Phase 2 procedure is composed of trial photolysis experiments at the chosen wavelength λ (313 or 366 nm) in an Ace-type PMGRR to determine the approximate rate constant and half-life of the test chemical and to choose the appropriate actinometer which has a rate constant approximately the same as the rate constant of the test chemical. First, an aqueous solution of test chemical at low optical density is photolyzed in the PMGRR at the chosen wavelength λ to determine k_{pc} and $(t_{1/2})_c$ using equations 3 and 5, respectively. If $(t_{1/2})_c$ is less than 12 hours, use the PNA/PYR actinometer. If $(t_{1/2})_c$ is greater than 12 hours, then use the PNAP/PYR actinometer. Trial photolysis experiments are then carried out at wavelength λ with the chosen low optical density actinometer to determine the molar concentration of pyridine needed to make the rate constant of the actinometer approximately match the rate constant of the test chemical.

In the Phase 3 procedure, low optical density aqueous solutions of test chemical and actinometer (at a fixed molar concentration of pyridine [PYR]) are photolyzed in identical tubes in the PMGRR at wavelength λ . Concentrations of test chemical and actinometer are measured as a function of time. These data are used in equation 6 to determine the ratio of the rate constants (k_{pc}/k_{pa}). The reaction quantum yield of the actinometer ϕ_a , employed in these experiments, can be determined at the molar concentration of pyridine [PYR] using equation 7 or 8. These data along with the molar absorptivity of the test chemical (ε_{313c} or ε_{366c}) and the actinometer (ε_{313a} or ε_{366a}) are substituted in equation 1 to determine the reaction quantum yield of the test chemical ϕ_c .

(2) Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method

For a low optical density test chemical (absorbance < 0.02) and for a high optical density actinometer (absorbance >2), such as the ferrioxalate actinometer, the reaction quantum yield of the test chemical ϕ_c at wavelength λ is given by the equation

$$\phi_c = \phi_a(k_{pc}/k_{pa})(2.303\varepsilon_{\lambda c}\ell)^{-1} , \qquad (9)$$

where ϕ_a is the reaction quantum yield of the ferrioxalate actinometer at wavelength λ, $\varepsilon_{\lambda c}$ is the molar absorptivity of the test chemical at wavelength λ, ℓ is the cell pathlength, k_{pc} is the first-order direct photolysis rate constant for the test chemical, and k_{pa} is the zero-order direct photolysis rate constant for the ferrioxalate actinometer. These rate constants are defined by the equations

$$\ell n(C_o/C_t)_c = k_{pc}t \qquad (10)$$

$$(C_t)_a = k_{pa}t\ , \qquad (11)$$

where $(C_o)_c$ and $(C_t)_c$ are the molar concentrations of the test chemical at time $t = 0$ and t and $(C_t)_a$ is the molar concentration of the ferrous ion formed at time t.

The laboratory procedure for determining the reaction quantum yield of the test chemical is divided into two phases using a uv-visible absorption spectrophotometer and a photochemical optical bench (POB) or a PMGRR containing a 450 watt medium pressure lamp with appropriate filters to isolate the monochromatic wavelength λ. In Phase 1, the molar absorptivities of test chemical (ε_{313c} and ε_{366c}) are determined using spectroscopic procedures outlined in Test Guidelines CG-1050 and CG-6000. Based on these results, photolysis experiments are carried out at 313 or 366 nm corresponding to the higher value of ε_{313c} or ε_{366c}.

In the Phase 2 procedure, aqueous low optical density test chemical solution (absorbance < 0.02) and aqueous high optical density solution of ferrioxalate actinometer (absorbance >2) are photolyzed sequentially in identical cells in POB or PMGRR at wavelength λ . In the first and third set of experiments, the ferrioxalate actinometer is photolyzed for a few minutes and the molar concentration of ferrous ion formed $(C_t)_a$ is measured as a function of time t. These data are used in equation 11 to determine an average value of the actinometer rate constant $(k_{pa})_{ave.}$. The second series of experiments involves the photolysis of the aqueous solution of test chemical in the POB or PMGRR in identical cells to those used in the actinometer experiments and the molar concentration of test chemical $(C_t)_c$ is measured as a function of the time t. These data are used in equation 10 to determine k_{pc}. Using k_{pc} and $(k_{pa})_{ave.}$, the molar absorptivity of the test chemical (ε_{313c} or ε_{366c}) , the pathlength of the cell ℓ , and the reaction quantum yield of the ferrioxalate actinometer ϕ_a at wavelength λ in equation 9, the reaction quantum yield of the test chemical ϕ_c can be determined.

As described in detail in Test Guideline CG-6000 and the associated Technical Support Document CS-6000, and briefly in Technical Support Document CS-6010, the direct sunlight photolysis of a chemical in an optically thin aqueous solution can be described by the following equations:

$$\ell n(C_o/C_t) = k_{pE}t \qquad (12)$$

$$t_{1/2E} = 0.693/k_{pE} \qquad (13)$$

$$k_{pE} = \phi_E k_a \ , \qquad (14)$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, $k_a = \Sigma k_{a\lambda}$, the sum of $k_{a\lambda}$ values for all wavelengths of sunlight that are absorbed by the chemical(i.e., the light absorption rate constant), t is the time, C_o and C_t are the molar concentrations of chemical at t = 0 and t, and $t_{1/2E}$ represents the half-life. The term k_{pE} represents the first-order direct photolysis rate constant for a chemical in a water body in sunlight in the units of reciprocal time. In general, the reaction quantum yield ϕ_E is equivalent to the reaction quantum yield ϕ_c determined in the laboratory.

Furthermore, under the same conditions cited above, the first-order direct photolysis rate constant, k_{pE}, is given by the equation

$$k_{pE} = \phi_E \Sigma \varepsilon_{\lambda c} L_\lambda \ , \qquad (15)$$

where ϕ_E is the reaction quantum yield, $\varepsilon_{\lambda c}$ is the molar absorptivity in the units $molar^{-1}\ cm^{-1}$, L_λ is the solar irradiance in water in the units 10^{-3} einsteins $cm^{-2}\ day^{-1}$, and the summation is taken over the range λ = 290 to 800 nm. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year. Solar irradiance data are tabulated in Test Guideline CG-6000, Tables 3-6, as a function of latitude and season of the year.

A simple screening test has been developed in Test Guideline CG-6000 using equation 15. As an approximation, it has been assumed that the reaction quantum yield ϕ_E is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 15 becomes

$$(k_{pE})_{max.} = \Sigma\varepsilon_{\lambda c}L_{\lambda} \quad . \qquad (16)$$

Using equation 16 in equation 13, the lower limit for the half-life is then given by

$$(t_{1/2E})_{min.} = 0.693/(k_{pE})_{max.} \quad . \qquad (17)$$

The molar absorptivity can be determined experimentally by the method outlined in Section II.B.1 and the solar irradiance data are tabulated in Tables 3-6 of Test Guidelines CG-6000. These data can then be used in equation 16 to calculate $(k_{pE})_{max.}$. Finally, $(k_{pE})_{max.}$ can be substituted in equation 17 to calculate $(t_{1/2E})_{min.}$. Based on these data, a decision can be made whether to determine the reaction quantum yield of the test chemical in the laboratory.

Once the reaction quantum yield has been determined in the laboratory by Procedures One or Two of this Test Guideline (CG-6010), it can be combined with the molar absorptivity data $\varepsilon_{\lambda C}$ and the appropriate L_λ values to calculate k_{pE} as a function of latitude and season of the year anywhere in the United States using equation 15. The corresponding half-life can be calculated using k_{pE} in equation 13.

D. Applicability and Specificity

For environmental photochemistry, the general procedures outlined in this Test Guideline are applicable to all chemicals which have uv-visible absorption maxima in the range 290-800 nm. Some chemicals have absorption maxima significantly below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, saturated alcohols, and saturated acids). This is a direct consequence of the Grotthus-Draper law of photochemistry. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals.

These test methods are applicable to pure chemicals and not to technical grade chemicals.

The molar absorptivity data ($\varepsilon_{\lambda C}$) obtained in Section II.B.1 can be combined with the appropriate L_λ data to estimate

$(k_{pE})_{max.}$ and $(t_{1/2E})_{min.}$ at shallow depths in water bodies as a function of latitude and season of the year in the U.S. If these data indicate that aqueous photolysis is a important process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.), then it is recommended that the reaction quantum yield ϕ_c be determined in the laboratory by Procedures One or Two outlined in this guideline. Once ϕ_c has been determined, it can be combined with the molar absorptivity data $\varepsilon_{\lambda c}$ and the appropriate L_λ data to calculate k_{pE} at shallow depths in water bodies as a function of latitude and season of the year in the United States. The corresponding half-life can then be calculated from k_{pE}.

Procedure One is only applicable to solutions of test chemicals and actinometers which have low optical densities (i.e., absorbance < 0.02). Procedure Two is only applicable to solutions of test chemicals with low optical densities and actinometers with high optical densities (i.e., absorbance >2).

Procedure one, as described in detail in this Test Guideline, is limited to the spectral region 290-400 nm because the recommended low optical density actinometers are only sensitive to light in this region. However, Procedure Two using the ferrioxalate actinometer is useful in the spectral region 290-500 nm. This procedure can be extended up to approximately 750nm using the Reinicke's salt actinometer [de Mayo and Shizuka (1976)].

There is a third procedure which could be used to determine ϕ_C in the laboratory. This procedure involves the determination of ϕ_C under the conditions when the test chemical and actinometer solutions both have high optical density. However, in general, this method is limited to only very soluble or strongly absorbing organic compounds. Thus, this method is not described in detail as a procedure in this Test Guideline. However, this method is briefly described in the associated Technical Support Document CS-6010 (Section II.E.3).

This Test Guideline has been designed to determine the molar absorptivity of a test chemical, $\varepsilon_{\lambda C}$, and its quantum yield, ϕ_C. These parameters can be used to determine environmental rate constants at low absorbance and shallow depths in pure water as a function of latitude and season of the year. Tables of solar irradiance (Table 3-6) have been included in Test Guideline CG-6000 to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp has written a GC SOLAR computer program to calculate the rates of photolysis as a function of depth in water, as a function of the attenuation (or absorption) coefficient of the water (α_λ) for natural water bodies, the average ozone layer thickness that pertains to the seasons and location of interest, and as a function of latitude and season of the year. The computer program is available on request. [R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.]

II. TEST PROCEDURES

A. Test Conditions

1. UV-Visible Absorption Spectrophotometer

The recommended uv-visible absorption spectrophotometer is described in Test Guideline CG-1050.

2. Special Photochemical Laboratory Equipment

There are a number of different designs of photochemical equipment which can be used to measure the reaction quantum yield in the laboratory and the one chosen will depend on the equipment available and to a certain extent on the light source and filter system chosen for the measurement. The apparatus chosen must contain a light source, appropriate filters, sample holders, and cells which allow solutions of test chemical and actinometer to be reproducibly irradiated with a uniform and constant amount of light at a discrete or narrow band wavelength λ. The temperature of the reaction cells must be at a reasonably constant temperature t_i ± 2°C in the range 20-30°C. The apparatus should be housed in a separate part of a laboratory and properly shielded so that laboratory personnel are not exposed to uv light and to exclude extraneous light that could contribute to the photoreaction of chemicals. If the ferrioxalate actinometer is used, then actinometry experiments must be carried out in a dark room with photographic "safe lights". Excellent reviews and descriptions of a wide variety of photochemical equipment used to measure the reaction quantum yield in the laboratory are given by Calvert and Pitts (1966), de Mayo and Shizuka (1976), Murov (1973), and Mill et al., (1982a) and these references are highly recommended.

a. Design of the Apparatus

i. Photochemical "Merry-Go-Round" Reactor

The design of the photochemical "merry-go-round" reactor (PMGRR) has been described in the literature [Moses et al. (1969), Murov (1973), de Mayo and Shizuka (1976)]. In the design of a PMGRR, the light source is in the center of the apparatus with reaction tubes arranged in a ring around the light source. The ring rotates around the light source to give a uniform irradiation of the reaction tubes, Figure 1A. Glass filters (f) may be inserted between the light source and the reaction tubes. Filter solutions (f) may also be contained in the immersion well holding the light source or in a glass donut that surrounds the light source. In order to dissipate the heat generated by the light source, the PMGRR may be immersed in a water bath or a stream of air may be passed through the space between the light source and the filters. The filter solutions can be circulated to an external cooling source. There are two types of PMGRR equipment; the Ace-type and the Moses-type and both are very similar. The Ace-type PMGRR is designed so that the entire reaction cell is irradiated while the Moses-type PMGRR [Moses et al. (1969)] is designed with windows (which act as slits with a fixed aperture) so that only a narrow portion of the reaction cell is irradiated. Since the Ace-type PMGRR is the only one commercially available, this Test Guideline has been specifically designed to use this PMGRR.

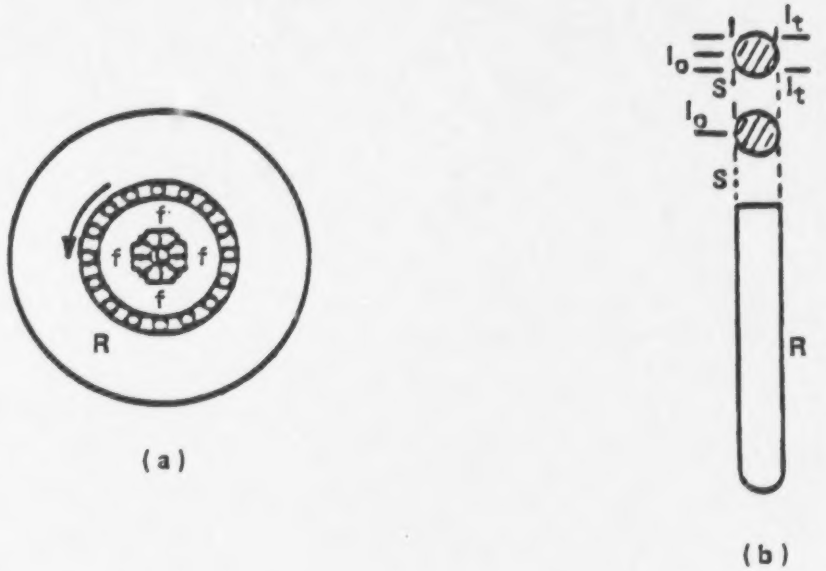

f
f
f
f
R
(a)
I_t
I_o
S
I_t
I_o
S
R
(b)

Figure 1A. (a) "Merry - Go - Round" Apparatus
(b) Cylindrical cell (R). I_o = incident light;
I_t = transmitted light passed through cell;
S = slit

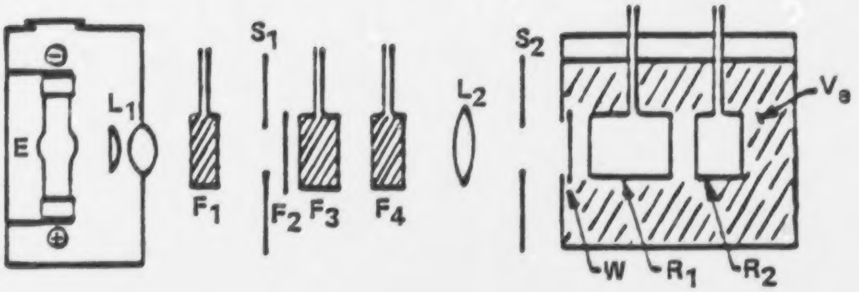

E
L_1
F_1
S_1
F_2
F_3
F_4
L_2
S_2
V_e
W
R_1
R_2

Figure 1B. A typical optical train, where E: point source arc;
L: lens; F: solution and glass filters; R_1: reaction cell;
R_2: actinometer cell; V_e: thermostat vessel; W: quartz window.

However, if the Moses-type PMGRR is available, this Test Guideline has to be slightly modified as described in Section II.J.11.c of the Technical Support Document CS-6010.

Small cylindrical tubes are used in the PMGRR to hold small volumes of test chemical and actinometer solutions. One tube is used for each datum point measured.

ii. Photochemical Optical Bench

The photochemical optical bench (POB) is composed of a light source, a lens, glass or chemical solution filters, and a reaction vessel. The component parts are mounted on a rail in the optical bench. A typical POB apparatus is depicted in Figure 1B. The light source is located at the end of the bench and contains a housing around a light source and a lens to collimate the beam. The light passes through glass filters and/or through cells containing filter solutions to transmit wavelength . Filter solutions in the cell may be circulated through a cooling system and the glass filters should be cooled with a stream of air to prevent heat buildup. The photochemical reaction vessel containing the test chemical solution or actinometer solution is mounted coaxially with the lamp on the bench so that the filtered light enters the window of the reaction vessel. The reaction vessel should be temperature controlled by circulating constant temperature water through side walls. The light flux is usually measured before and after the photolysis of the test chemical solution. Commercially available optical benches are highly recommended but simple "home-made" benches can be used [Andre (1977)]. If a "home-made" bench is used, it must be completely described.

b. Light Sources

There are a number of light sources which are available for use in photochemical studies: for example, low, medium, and high pressure mercury lamps; xenon lamps; and lasers. The characteristics and application of these lamps are described in detail by Calvert and Pitts (1966), Murov (1973), and de Mayo and Shizuka (1976). The use of a POB or PMGRR will often narrow the choice of lamp with regard to use of a point source or a tube-type lamp since the former can be focused to give a collimated beam while the latter cannot. The light source should emit light at a constant and high intensity. The 450 watt medium pressure mercury lamp is highly recommended for use in this Test Guideline.

c. Light Filtering Systems

Monochromatic or narrow band wavelength light is essential for the accurate determination of the reaction quantum yield of a test chemical. Various systems for isolating monochromatic or narrow band wavelengths are described by Calvert and Pitts (1966), Murov (1973), and de Mayo and Shizuka (1976). Two filter systems commonly used to isolate the 313 and 366 bands from a 450 watt medium pressure mercury lamp are highly recommended and have been incorporated in this Test Guideline. These two filter systems are described as follows:

(1) 313 nm filtering system: Corning Glass CS-754 filter* with 0.005 M potassium chromate solution containing 3% sodium carbonate.

(2) 366 nm filtering system: Corning Glass CS 0-52 and CS 7-60 filters.

Reagent grade chemicals should be used to prepare the chemical filter solution. Since this filter solution (and in general any filter solution) degrades slowly over prolonged periods of photolysis, the solution should be carefully monitored. Even when tap water is used to cool the lamp, the buildup of solid material or algae may reduce the light intensity; and this must be checked repeatedly.

d. Reaction Cells

In general, reaction cells of large volume are appropriate for POB equipment while small reaction cells are used for PMGRR equipment. These cells should be constructed of borosilicate glass or quartz. Actinometer and test chemical solutions must be photolyzed in identical cells and should contain the same volumes of actinometer and test chemical solution.

---

*If the test chemical does not absorb light at wavelengths greater than 400 nm, this filter is not needed. The 754 filter is designed to block out visible light.

For the PMGRR equipment, disposable culture tubes (13 x 100 mm) with Teflon-lined screw caps or quartz tubes (13 x 100 mm) with quartz ground glass stoppers or borosilicate screw caps, Teflon-lined, are recommended for use as reaction tubes. Grease should be avoided since hydrophobic chemicals might adsorb to it. In carrying out the photolysis experiments, one tube is used for each datum point measured. The pathlength of these cells is discussed in Section II.A.3.

For the POB equipment, the most common and functional design for reaction cells is a cylindrical shape with optically flat circular windows fused to each end of the cylinder and at right angles to its axis. The windows should be made of material that will transmit 100% of the light at the desired wavelengths. Optically flat quartz windows are recommended. The size of the cylindrical reaction cell and windows should be consistent with the dimensions of the light beam used in the equipment. The reaction cell should be of sufficient volume to permit removal of samples for analysis without significantly altering the volume of the reaction solution in the cell. The cell pathlength can be measured directly or it can be determined by the procedure discussed in Section II.A.3. Details for the construction of these reaction cells may be found in Calvert and Pitts (1966) and de Mayo and Shizuka (1976).

3. Cell Pathlength

Zepp (1978) described an experimental method using an isolated wavelength band to determine the effective pathlength of any cell. This procedure is described in the associated Technical Support Document CS-6010, Section II.D). This procedure has been used to measure the effective pathlength of Corning Glass culture tubes 13 x 100 mm and it was found that ℓ was 11.2 mm [Mill et al. (1982a)]. In a similar manner, reaction tubes made from borosilicate glass stock of o.d. 12 mm had an effective pathlength of 10.0 mm [Mill et al. (1982a)]. This procedure can be used to measure the pathlength of cylindrical cells designed for the POB apparatus. However, it is recommended that the pathlength of the cylindrical cells be measured directly with a precise centimeter ruler or an equivalent measuring device.

4. Solvents

If the half-life of an aqueous solution of test chemical in the photochemical equipment is less than 24 hours, then distilled water meeting ASTM Type II standards, or an equivalent grade, is recommended for use in this Test Guideline. If the half-life of an aqueous solution of test chemical in the photochemical equipment is greater than 24 hours, then water meeting ASTM type IIA standards, or an equivalent grade, is highly recommended for use in this Test Guideline to minimize biodegradation. ASTM Type II and IIA water are described in ASTM D 1193-77. Air saturated water is required for photolysis of test chemical solutions. Air saturated water can be easily prepared by allowing the ASTM Type II water to equilibrate in a vessel plugged with cotton or ASTM Type IIA water to equilibrate in a vessel plugged with sterile cotton.

Reagent grade acetonitrile is recommended as the organic cosolvent with water in photochemical studies. Spectrograde acetonitrile or methanol is recommended for spectroscopic studies to determine the molar absorptivity of the test chemical.

5. Sterilization

If the half-life of an aqueous solution of test chemical in the photochemical equipment is greater than 24 hours, then it is important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions for photolysis studies in order to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable non-chemical method.

6. pH Effects

It is recommended that all photolysis and molar absorptivity experiments be carried out at pHs at least two pH units above the pK_a and at least two pH units below the pK_a for any chemical that ionizes or protonates (e.g., carboxylic acids, phenols, and amines). Buffers described in Section II.B.1 should be used.

7. Volatile Chemical Substances

Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels. Disposable culture tubes with Teflon-lined screw caps or quartz tubes with quartz or borosilicate screw caps, Teflon-lined, are recommended. Grease should not be used. Volatile compounds can be conveniently studied in culture tubes equipped with gas-tight Mininert® valves. Samples can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate. As an alternative, the tubes can be sealed with a torch. In addition, the reaction vessels should be as completely filled as is possible to minimize volatilization to any air space.

8. Control Solution

It is extremely important to take certain precautions to prevent loss of chemical from the reaction vessels by processes other than photolysis. For example, biodegradation and volatilization can be eliminated or minimized by use of sterile conditions and minimal air space in sealed vessels. Hydrolysis is a process which cannot be minimized by such techniques. Thus, control vessels containing test substance which are not exposed to light are required. In this way, the loss of test chemical in processes other than photolysis may be determined and eliminated or minimized. For simplicity, if the loss of chemical in the control is small (i.e., approximately 10% or less), one can calculate a first-order loss, k_{loss}, and subtract it from $(k_p)_{obs}$. to give the corrected direct photolysis rate constant k_p. If hydrolysis is found to be significant (greater than 10%), hydrolysis studies should be carried out first. [Test Guideline CG-5000].

9. Absorption Spectrum as a Criterion for Performing the Reaction Quantum Yield Experiments

This aqueous photolysis test is applicable to all chemicals which have uv-visible absorption maxima in the range 290-800 nm. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals to determine the reaction quantum yield. The absorption spectrum of the chemical in aqueous solution can be measured by the procedures given in Test Guideline CG-1050.

10. Actinometers

Chemical actinometers are used in reaction quantum yield experiments to measure the integrated light intensity incident on the sample during photolysis. Chemical actinometers have photochemical reactions which have well-defined reaction quantum yields ϕ_a at wavelength λ .

a. Low Optical Density Actinometers

Two low optical density actinometers (absorbance <0.02) are described which can be used to determine the reaction quantum yield of a test chemical at low optical density (absorbance < 0.02) in Procedure One of this Test Guideline. These actinometers are: (i) p-nitroacetophenone-pyridine actinometer (PNAP/PYR); and (ii) p-nitroanisole-pyridine actinometer (PNA/PYR). The rate constants and half-lives of these actinometers can be adjusted to match the rate constant and half-life of the test chemical by adjusting the concentration of pyridine. With a 450 watt medium pressure mercury lamp in an Ace-type PMGRR, the PNAP/PYR actinometer can be adjusted with pyridine to have half-lives that range from greater than 12 hours to several weeks while the PNA/PYR actinometer can be adjusted with pyridine to have half-lives that range from approximately 15 minutes to approximately 12 hours.*

i. p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR)

The reaction quantum yield of the PNAP/PYR actinometer is a function of the molar concentration of pyridine [PYR] and this relationship is given by equation 7. This equation is valid up to 0.2 M pyridine. The initial concentration of PNAP (C_o) is precisely set in the range of approximately 1×10^{-5} M. The molar absorptivities of PNAP at 313 and 366 nm are: $\varepsilon_{313a} = 2{,}056\ M^{-1}\ cm^{-1}$; and $\varepsilon_{366a} = 160\ M^{-1}\ cm^{-1}$. The chemical analysis of PNAP is given in Section II.A.11.b.

---

*If a Moses-type PMGRR is used, then new criteria have to be defined to determine which of these two actinometers should be used. For details see Section II.J.11.c of the Technical Support Document CS-6010.

Trial photolysis experiments are required to determine the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical (within ± 50%). In the Phase 2 experiments of Procedure One, the rate constant of the test chemical k_{pc} is determined in the Ace-type PMGRR with a 450 watt medium pressure mercury lamp. As an approximation, for the PNAP/PYR actinometer in the PMGRR with a 450 watt medium pressure mercury lamp, the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical at 313 and 366 nm is given by the following equations:

at 313 nm $$[PYR] = 1.16\ k_{pc} \tag{18}$$

at 366 nm $$[PYR] = 5.95\ k_{pc}\quad , \tag{19}$$

where k_{pc} is in the units of $(\text{hours})^{-1}$ and [PYR] is the molar concentration of pyridine.

A trial experiment is then carried out in the Phase 2 procedure by preparing the actinometer with the molar concentration of pyridine [PYR] estimated from equations 18 or 19 and photolyzing the actinometer solution at a fixed molar concentration of PNAP (C_o) in the PMGRR at 313 or 366 nm. After approximately 50% transformation, the concentration of PNAP (C_t) is recorded at time t and an approximate rate constant k_{pa} is calculated using these data in equation 2. This trial experiment is repeated by adjusting the molar concentration of pyridine [PYR] until the rate constant of the test chemical and actinometer are approximately equal.

ii. p-Nitroanisole-Pyridine Actinometer (PNA/PYR)

The reaction quantum yield of the PNA/PYR actinometer is a function of the molar concentration of pyridine [PYR] and this relationship is given by equation 8. This equation is valid up to 0.02 M pyridine. The initial concentration of PNA (C_o) is precisely set in the range of approximately 1×10^{-5} M at 366 nm and at approximately 0.4×10^{-5} M at 313 nm. The molar absorptivities of PNA at 313 and 366 nm are: $\varepsilon_{313a} = 10{,}300\ M^{-1}\ cm^{-1}$; and $\varepsilon_{366a} = 1{,}990\ M^{-1}\ cm^{-1}$. The chemical analysis for PNA is given in Section II.A.11.c.

Trial photolysis experiments are required to determine the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical (within ± 50%). In the Phase 2 experiments of Procedure One, the rate constant of the test chemical k_{pc} is determined in an Ace-type PMGRR with a 450 watt medium pressure mercury lamp. As an approximation, for the PNA/PYR actinometer in the PMGRR with a 450 watt medium pressure mercury lamp, the concentration of pyridine needed to adjust the rate constant of the actinometer to equal the rate constant of the test chemical at 313 and 366 nm is given by the following equations:

$$\text{at 313 nm} \quad [\mathrm{PYR}] = 8.93 \times 10^{-3} (k_{pc} - 0.0722) \qquad (20)$$

$$\text{at 366 nm} \quad [\mathrm{PYR}] = 1.85 \times 10^{-3} (k_{pc} - 0.0349) \quad , \qquad (21)$$

where k_{pc} is in the units of $(\text{hours})^{-1}$ and [PYR] is the molar concentration of pyridine.

A trial experiment is then carried out in the Phase 2 procedure by preparing the actinometer with the molar concentration of pyridine [PYR] estimated from equation 20 or 21 and photolyzing the actinometer solution at a fixed molar concentration of PNA (C_o) in the Ace-type PMGRR at 313 or 366 nm. After approximately 50% transformation, the concentration of PNA (C_t) is recorded at time t and an approximate rate constant k_{pa} is calculated using these data in equation 2. This trial experiment is repeated by adjusting the molar concentration of pyridine [PYR] until the rate constant of the test chemical and actinometer are approximately equal.

b. High Optical Density Actinometer
(Ferrioxalate Actinometer)

The ferrioxalate actinometer at high optical density (absorbance > 2) is a widely used actinometer by photochemists and has been recommended for use in Procedure Two [Murov (1973), de Mayo and Shizuka (1976), Calvert and Pitts (1966)]. This actinometer is applicable over the environmentally relevant range 290-500 nm. The net photochemical reaction is

$$Fe^{3+} + (C_2O_4{}^{2-})/2 \xrightarrow{h\nu} Fe^{2+} + CO_2 \quad . \qquad (22)$$

At high optical density, the reaction kinetics are zero-order and the kinetics are followed by measuring the molar concentration of Fe^{2+} formed as a function of time t using equation 11. The Fe^{2+} formed is measured by procedures outlined in Section II.A.11.d. Murov (1973) gives a detailed procedure for using this actinometer and it is highly recommended. This procedure should be modified slightly to use the ferrioxalate at 0.15 M. The irradiated solution is diluted 100-fold prior to analysis for ferrous ion, which must not be allowed to exceed 0.005 M. Table 1 of the associated Technical Support Document CS-6010 lists the reaction quantum yield as a function of wavelength. At 0.15 M, the quantum yield (ϕ_a) at 313 and 366 nm is 1.20 and 1.18, respectively. All ferrioxalate actinometry experiments must be carried out in a dark room with photographic "safe lights." Ferrioxylate is available from Alfa Inorganics. If the test chemical absorbs light at wavelengths greater than 500 nm, then Reinicke's salt can be used as the actinometer. The use of Reinicke's salt actinometer is described in Technical Support Document CG-6010, Section II.C.2.c.

11. Chemical Analysis of Solutions

a. Chemical Analysis of Test Chemical Solutions

In determining the concentration of the chemical in solution, an analytical method should be selected which is most applicable to the analysis of the specific chemical substance. Chromatographic methods are generally recommended because of their chemical specificity in analyzing the parent chemical substance without interference from impurities. Whenever practicable the chosen analytical method should have a precision of ± 5 percent or better.

b. Chemical Analysis of p-Nitroacetophenone (PNAP)

The p-nitroacetophenone (PNAP) in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 2.5% acetic acid, 50% acetonitrile, and 47.5% water which is passed through the column at a flow rate of 2 mL/minute. [Mill et al. (1982) and Mill and Dulin (1982)].

c. Chemical Analysis of p-Nitroanisole (PNA)

The p-nitroanisole (PNA) in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 50% acetonitrile and 50% water which is passed through the column at a flow rate of 2 mL/minute [Dulin and Mill (1982)].

d. Chemical Analysis of Ferrous Ion in the Ferrioxalate Actinometer

The concentration of Fe^{2+} formed in the photolysis of the ferrioxalate actinometer is measured spectrophotometrically via the formation of a red phenanthroline complex and determining the absorbance of the complex at 510 nm. Murov (1973) describes a detailed procedure for measuring the molar concentration of ferrous ion formed in the photolysis reaction and this procedure is highly recommended. This procedure has been modified slightly to use the ferrioxalate at 0.15 M. As a result, the irradiated solution has to be diluted 100-fold prior to analysis for ferrous ion, which must not be allowed to exceed 0.005 M.

B. Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method

1. Phase 1 Experiments: UV-Visible Absorption Spectra

The uv-visible absorption spectra in aqueous solution can be determined by the methods described in Test Guidelines CG-1050 and CG-6000. It is recommended that the following additional procedures be followed:

(a) For chemicals which ionize or protonate (e.g., carboxylic acids, phenols, amines), carry out uv-visible absorption studies at pHs at least two pH units above the pK_a and at least two pH units below the pK_a. Prepare buffer solutions at 25°C using reagent grade chemicals and distilled water as follows:

pHs in the range 3-6*: NaH_2PO_4 , HCl

pHs in the range 6-8*: KH_2PO_4 , NaOH

pHs in the range >8: Prepare buffers as described in the Handbook of Chemistry and Physics.

Check the pH of all the buffer solutions with a pH meter at 25°C and adjust to the proper pH, if necessary. These buffer solutions can then be added to the test chemical solution until the desired pH is obtained. If these buffers are inadequate, then adjust the pH of the test chemical solution with 1 M HCl or NaOH at 25°C.

(b) Measure the absorbance, $A_{\lambda c}$, as a function of wavelength in the range 290-800 nm in duplicate. If applicable, measure $A_{\lambda c}$ at each experimental pH [Section II.A.6, p.26].

---

*Use the minimum concentration of buffers to attain the desired pH.

Record, in duplicate, the baseline where both the sample and reference cells are filled with blank solutions. These data will be used to calculate the molar absorptivities for the appropriate intervals and wavelength centers, Table 1, Test Guideline CG-6000, where the test chemical absorbs light. The wavelength center is defined as the midpoint of the interval range listed in Table 1.

Measure $A_{\lambda c}$ at 313.0 nm and 366.0 nm in duplicate. These data will be used to calculate the molar absorptivities of the test chemical, ε_{313c} and ε_{366c}. Photolysis experiments should be carried out at the wavelength corresponding to the higher value of the molar absorptivity.

It must be emphasized that the molar absorptivities of the test chemical $(\varepsilon_{\lambda c})$ must be carefully determined, espec[illegible]y in the tails of the absorption bands at $\lambda \geqslant 290$ nm. Large errors will occur when calculating photolysis rate constants and half-lives if these measurements are not carefully carried out.

2. Phase 2 Experiments: Trial Photolysis Experiments

a. Determination of the Approximate Rate Constant of the Test Chemical

i. Preparation of Buffer Solutions

Prepare buffer solutions according to the procedures outlined in Section II.B.1 (p. 35) using reagent grade chemicals and pure water as described under Test Conditions, Section II.A.4 (p.25), for chemicals that reversibly ionize or protonate.

ii. Preparation of Test Chemical Solution

Prepare a homogeneous solution of test chemical below its water solubility and at an absorbance less than 0.02 in the photolysis reaction vessel at 313 or 366 nm. For very hydrophobic chemicals, it is difficult and time consuming to prepare aqueous solutions. To facilitate the preparation of aqueous solutions containing very hydrophobic chemicals and to allow for easier analytical procedures, the following procedure may be used as an aid in the dissolution of the test chemical. Dissolve the pure test chemical in reagent grade acetonitrile. Add pure water as described under Test Conditions, Section II.A.4 (p. 25), or buffer solution, as described in Section II.B.1 (p. 35), for chemicals which ionize or protonate, to an aliquot of the acetonitrile solution. Do not exceed one-volume percent of acetonitrile in the final solution.

iii. Performance of the Test

Prepare an aqueous solution of test chemical as described in Section II.B.2.a.ii (p. 38) and a sufficient number of samples in quartz or borosilicate glass tubes (Section II.A.2.d, p. 23) to perform all the required tests. Measure the initial concentration of test chemical (C_o) as described in Section II.A.11.a (p. 34) in duplicate. Fill the tubes as completely as possible and seal them. Do not use grease. Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminum foil or by any other suitable method [Section II.A.8, p.27].

Place the samples, including the controls, in the Ace-type PMGRR with a 450 watt medium pressure mercury lamp and appropriate filters as described in Section II.A.2.a.i (p. 19) and II.A.2.b, c (p. 22). The reaction tubes should be controlled to a temperature of t_i ± 2°C within the range 20 - 30°C. Photolyze the samples at 313 or 366 nm corresponding to the higher value of the molar absorptivity of the test chemical (ϵ_{313c} or ϵ_{366c} determined in the Phase 1 experiments, Section II.B.1, p. 35) until approximately 50% of the test chemical has transformed. Measure the molar concentration of the test chemical (C_t) in duplicate at time t. For test chemicals that ionize or protonate, carry out the photolysis experiments at the required pHs as described under Test Conditions, Section II.A.6 (p. 26).

After the photolysis experiments are completed, determine the concentration of test chemical in the controls in duplicate. If a significant loss of test chemical has occurred in the controls, determine the cause and eliminate, or minimize, the loss. If hydrolysis is found to be significant, hydrolysis experiments should be carried out first [Section II.A.8, p. 27].

The data obtained in this section will be used to determine an approximate k_{pc} and $(t_{1/2})_c$ using equations 3 and 5, respectively.

b. Actinometry Experiments

These experiments are designed to choose the appropriate low optical density actinometer [i.e., PNAP/PYR, Section II.A.10.a.i (p. 29) or PNA/PYR, Section II.A.10.a.ii, p. 31] and to adjust the rate of the chosen actinometer so that the rate constant of the actinometer (k_{pa}) is approximately equal to the rate constant of the test chemical (k_{pc}). Based on the photolysis experiments in Section II.B.2 (p. 37), if $(t_{1/2})_c$ is less than 12 hours, use the PNA/PYR actinometer; if $(t_{1/2})_c$ is greater than 12 hours, use the PNAP/PYR actinometer.

i. Preparation of Actinometer Solutions

PNAP/PYR Actinometer: Using the test chemical photolysis rate constant k_{pc} determined in Section II.B.2.a (p. 37) and equations 18 or 19, determine the molar concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical at the appropriate wavelength 313 or 366 nm (chosen based on the results of the Phase 1 experiments). Once the molar concentration of pyridine [PYR] has been estimated, the actinometer solution can be prepared as follows. Dissolve 0.0165 grams of PNAP in 10 mL of acetonitrile (0.01 M). Add 1 mL of this solution to a one liter volumetric flask. Add to the volumetric flask the mass in grams, or the volume V of pyridine at 20°C, obtained from the equations

$$\text{mass (grams)} = 79.1\,[\text{PYR}] \qquad (23)$$

$$V(\text{mL}) = 80.6[\text{PYR}] \qquad (24)$$

Fill the volumetric flask with pure water (Section II.A.4, p. 25) to give one liter of solution and shake vigorously to make sure that the solution is homogeneous. The resultant concentration of PNAP is 1.00×10^{-5} M. The PNAP/PYR solution should be wrapped with aluminum foil and kept from bright light.

PNA/PYR Actinometer: Using the test chemical photolysis rate constant k_{pc} determined in Section II.B.2.a (p. 37) and equations 20 or 21, determine the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical at the appropriate wavelength 313 or 366 nm (chosen based on the results of the Phase 1 experiments). Once the molar concentration of pyridine has been determined, an actinometer solution can be prepared as follows. If photolysis experiments are to be carried out at 366 nm, dissolve 0.0153 grams of PNA in 10 mL of acetonitrile (0.01 M). Add 1 mL of this solution to a one liter flask. Add to the volumetric flask the mass in grams, or the volume of pyridine at 20°C as defined by equations 23 and 24. Fill the volumetric flask with pure water (Section II.A.4, p. 25) to give one liter of solution and shake vigorously to make sure that the solution is homogeneous. The resultant concentration is 1.00×10^{-5} M. The PNA/PYR solution should be wrapped with aluminum foil and kept from bright light.

If photolysis experiments are to be carried out at 313 nm, dissolve 0.00612 grams of PNA in 10 mL of acetonitrile (0.004 M). Then follow the procedure described above. The resultant PNA concentration is 0.400×10^{-5} M. The PNA/PYR solution should be wrapped with aluminum foil and kept from bright light.

ii. Performance of the Test

Prepare a solution of the appropriate actinometer as described in Section II.B.2.b.i (p. 40). Measure the initial molar concentration of actinometer C_o[PNAP or PNA as described in Sections II.A.11.b, c (p. 34)]. Prepare a sufficient number of samples in borosilicate or quartz tubes [Section II.A.2.d (p. 23)] to perform all the required tests. The tubes should be identical to the tubes used to determine k_{pc} (Section II.B.2.a.iii, p. 38). Fill the tubes as completely as possible and seal them. It is important that the actinometer tubes contain the same volume as that used in the test chemical tubes (Section II.B.2.a.iii, p. 38). Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminimum foil or by any other suitable method [Section II.A.8, p. 27]. Place the samples, including the controls, in the Ace-type PMGRR with the 450 watt medium pressure mercury lamp and the appropriate filters. The tubes should be controlled to a temperature $t_i \pm 2°C$. Photolyze the samples at the chosen wavelength λ (313 or 366 nm) until approximately 50% of the actinometer has transformed. Determine the concentration of the actinometer (C_t) in duplicate by the appropriate procedure described in Section II.A.11.b, c (p. 34) at time t. Calculate a rate constant using equation 2. If the rate constant k_{pa} is not approximately the same as the rate constant of the test chemical k_{pc}, then repeat the experiment with an appropriate concentration of pyridine until k_{pa} is approximately equal to k_{pc} (within ± 50%).

3. Phase 3 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical

Based on the results of the Phase 1 and 2 experiments, use the appropriate actinometer with the required concentration of pyridine to make the rate constant of the actinometer aproximately match the rate constant of test chemical. Follow the procedure outlined in Section II.B.2.b.i (p. 40) to prepare the actinometer and a sufficient number of samples in borosilicate glass or quartz tubes [Section II.A.2.d (p. 23)] to perform all the required tests. Determine the initial concentration of the actinometer $(C_o)_a$ in duplicate according to the appropriate procedure in Section II.A.11 (p. 34). Fill all the tubes as completely as possible, seal them, and cover half of the tubes with aluminum foil as soon as possible after preparation. Prepare an aqueous solution of test chemical as described in Section II.B.2.a.ii (p. 38) and determine the initial concentration of test chemical $(C_o)_c$ in duplicate according to the procedure described in Section II.A.11.a (p. 34). Prepare a sufficient number of samples in borosilicate or quartz tubes to perform all the required tests. Fill all the tubes as completely as possible, seal them, and cover half of the tubes with aluminum foil as soon as possible after preparation. The reaction tubes for actinometer and test chemical must be identical and contain the same volume of solution. Place the tubes to be photolyzed in the Ace-type PMGRR containing a 450 watt medium pressure mercury lamp and the appropriate filters, Sections II.A.2.b,c (p. 22), and all the control tubes close to the PMGRR. The tubes should be temperature controlled to a temperature $t_i \pm 2°C$. Based on the results of the Phase 2 experiments, determine the concentration of test chemical and actinometer periodically between 10 and 80% transformation in duplicate [at least 6 data points at approximately equal times]. Determine the concentration of actinometer and test chemical in the controls in duplicate at each time point.

C. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method

1. Phase 1 Experiments: UV-Visible Absorption Spectra

Follow the procedure outlined in Section II.B.1 (p. 35) and measure the absorbance of the test chemical, $A_{\lambda c}$, as a function of wavelength in the range 290-800 nm in duplicate. Measure $A_{\lambda c}$ at 313.0 and 366.0 nm in duplicate. The absorbance data at 313.0 and 366.0 nm will be used to calculate the molar absorptivities of the test chemical, ϵ_{313c} and ϵ_{366c}, and photolysis experiments should be carried out at the wavelength corresponding to the highest value of the molar absorptivity.

2. Phase 2 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical

a. Preparation of Buffer Solutions

Prepare buffer solutions according to the procedures outlined in Section II.B.2.a.i (p. 37) for a test chemical which ionizes or protonates.

b. Preparation of Test Chemical Solution

Prepare a homogeneous solution of test chemical according to the procedures outlined in Section II.B.2.a.ii (p. 38).

c. Preparation of Ferrioxalate Actinometer Solution

Prepare a ferrioxalate actinometer solution at 0.15 M as described in Section II.A.10.b (p. 33) following the procedure outlined by Murov (1973) which has been modified slightly. Since the ferrioxalate actinometer solution is very sensitive to visible light, all these actinometer experiments must be carried out in a dark room with photographic "safe lights."

d. Determination of the Quantum Yield of the Test Chemical

Prepare a solution of the ferrioxalate actinometer at high optical density (absorbance > 2) as described in Section II.C.2.c (p. 45) and completely fill the reaction cell described in Section II.A.2.d (p. 23). Place the reaction cell in the photochemical optical bench (POB) as described in Section II.A.2.a.ii (p. 21) with a 450 watt medium pressure mercury lamp (Section II.A.2.b, p. 22) and appropriate filters (Section II.A.2.c, p. 22). The reaction cell should be controlled to a temperature of $t_i \pm 2°C$ in the range 20 - 30°C. The filter system used should isolate the wavelength 313 or 366 nm corresponding to the larger value of ε_{313c} or ε_{366c} determined in the Phase 1 experiments. Photolyze the actinometer solution in the reaction cell for 1, 3, 6, 9, 12, and 15 minutes and measure the concentration of Fe^{2+} formed spectrophotometrically by the procedure outlined in Section II.A.11.d (p. 35) in duplicate at each of the time points. No time points should be taken when the optical density of the actinometer falls below 2. Only withdraw small volumes for analysis so that the total volume of the actinometer solution in the reaction cell does not change appreciably. This procedure is repeated in a third set of experiments soon after the test chemical photolysis experiments are performed.

In the second set of experiments, a solution of test chemical is prepared according to the procedure outlined in

Section II.C.2.b (p. 45) at low optical density (absorbance <0.02). Measure the initial concentration $(C_o)_c$ in duplicate by the procedure outlined in Section II.A.11.a (p. 34). Fill two reaction cells with the same volume as that used in the actinometer experiments. One of these cells is wrapped with aluminum foil, placed close to the POB and temperature controlled to t_i ± 2°C. The second reaction cell (which is the same one used for the actinometry experiments) is placed in the POB and photolyzed at t_i ± 2°C at the same wavelength used in the actinometry experiments. Determine the concentration of test chemical periodically between 10 and 80% transformation in duplicate [at least 6 data points at approximately equal times]. Only withdraw small volumes for analysis so that the total volume of the test chemical solution in the reaction cell does not change appreciably. Determine the concentration of the control in duplicate at each time point.

As an alternative procedure, the same series of experiments described above can be carried out in a PMGRR using individual reaction tubes for each datum point.

III. DATA AND REPORTING

A. Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method

1. Phase 1 Experiments: UV-Visible Absorption Spectra

a. Treatment of Results

The molar absorptivity $(\varepsilon_{\lambda c})$ can be determined from the absorption spectra using the expression

$$\varepsilon_{\lambda c} = A_{\lambda c}/C\ell \quad , \tag{25}$$

where $A_{\lambda c}$ is the absorbance at wavelength λ, C is the molar concentration of test chemical, ℓ is the cell pathlength in centimeters. The molar absorptivity of the chemical should be determined for the wavelengths listed in Table 1, Test Guideline CG-6000, for a solution of concentration C and in a cell with pathlength ℓ . If the absorption curve is flat within the interval around the wavelength center, $\varepsilon_{\lambda c}$ may be determined from the absorbance $A_{\lambda c}$ at λ center using equation 25. If a large change in absorbance occurs within this interval, obtain an average $A_{\lambda c}$ at λ center based on the two absorbances at the boundary of the interval. Calculate an average $\varepsilon_{\lambda c}$ using the average value of $A_{\lambda c}$ in equation 25. Determine the molar absorptivity for each replicate and calculate a mean value.

Determine the molar absorptivity of the test chemical at 313.0 and 366.0 nm for a solution of test chemical at a molar concentration C and in a cell of pathlength ℓ using equation 25. Determine $A_{\lambda c}$ and the molar absorptivity at 313.0 and 366.0 nm for each replicate and calculate a mean value.

Using the molar absorptivities obtained from the spectra and the values of L_λ from Table 3 to 6 of Test Guideline CG-6000, calculate the maximum direct photolysis rate constant $(k_{pE})_{max.}$ at a specific latitude (corresponding to the manufacturing site) and season of the year using equation 16. The corresponding minimum half-life, $(t_{1/2E})_{min.}$ can then be calculated using this $(k_{pE})_{max.}$ in equation 17.

b. Test Data Report

(1) Report the name, structure, and purity of the test chemical.

(2) Submit a recent test spectrum on appropriate reference chemicals for photometric and wavelength accuracy.

(3) Submit the original chart, or photocopy, containing a plot of absorbance of test chemical vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10 nm intervals. Each spectrum should be clearly marked with the test conditions.

(4) Report the concentration of the test chemical solution, the type of absorption cell used (quartz or borosilicate glass) and the pathlength.

(5) Report $A_{\lambda c}$ and $\epsilon_{\lambda c}$ at λ center for each replicate and the mean value.

(6) Report the identity and composition of the solvent used in the spectral absorption study.

(7) Report ε_{313c} and ε_{366c} along with A_{313c} and A_{366c} for each replicate and the mean value.

(8) Report $(k_{pE})_{max.}$ and $(t_{1/2E})_{min.}$ for the summer and winter solstices using the appropriate L_{λ} values from Tables 3-6, Test Guideline CG-6000, closest to the latitude of the chemical manufacturing site.

(9) For chemicals that ionize or protonate, report the data for steps 1-8 at the required pHs [Section II.A.6 (p. 26)].

(10) For a chemical that ionizes or protonates, report its pK_a. Report the type and concentration of the buffers employed for each pH.

(11) Describe the method employed in determining the test chemical's concentration.

(12) Report the name and model of the spectrophotometer used.

(13) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, gain, etc.

(14) If a Moses-type PMGRR was used in these experiments, describe it completely and report the aperture in cm^2.

2. Phase 2 Experiments: Trial Photolysis Experiments

a. Determination of the Approximate Rate Constant of the Test Chemical

i. Treatment of Results

From the photolysis experiments carried out at 313 or 366 nm (photolysis wavelength chosen based on the results of the Phase 1 experiments), use the concentration $(C_t)_c$ corresponding to approximately 50% of the initial molar concentration of chemical remaining at time t along with the initial molar concentration $(C_o)_c$ and the time t in hours in equation 3 to calculate k_{pc} in hours^{-1}. From the analysis of the two samples at time t = 0 and t, calculate a mean value of $(C_o)_c$ and $(C_t)_c$, and a value of k_{pc}. If a slight loss of chemical has been detected in the controls, then calculate a rate constant as follows. Calculate an average concentration $(C_t)_c$ based on duplicate measurements of concentration in the controls at the end of the experiment (time t). Use this concentration along with the average initial concentration $(C_o)_c$ and t in equation 3 and calculate a rate constant k_{loss}. Using this rate constant along with the observed rate constant in the photolysis experiments, the corrected rate constant is then

$$k_{pc} = (k_{pc})_{obs.} - k_{loss} \quad . \qquad (26)$$

Calculate the half-life, $(t_{1/2})_c$ using the corrected value of k_{pc} in equation 5.

ii. Specific Analytical and Recovery Procedures

(1) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision; and

(2) if extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

iii. Other Test Conditions

(1) Report the size, approximate cell wall thickness, pathlength ℓ , and type of glass used for the test chemical reaction tubes. If the cell pathlength was measured by the procedure described in the Technical Support Document, Section II.D, report all the data obtained in these experiments.

(2) Report the initial pH of all test solutions, if appropriate.

(3) If acetonitrile was used to solubilize the test substance, report the percent, by volume.

(4) If a significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

iv. Test Data Report

(1) Report the wavelength used to photolyze the test chemical.

(2) Report the initial molar concentration of test chemical $(C_o)_c$ of each replicate and the mean value.

(3) Report the molar concentration of test chemical $(C_t)_c$ for each replicate and the mean value. Report the time t.

(4) Report the molar concentration of each replicate control sample and the mean value after completion of the experiment. Report the time t.

(5) Report the value of k_{pc} and $(t_{1/2})_c$. If small losses of chemical were observed, report $(k_{pc})_{obs.}$, k_{loss}, and k_{pc}. Report the half-life $(t_{1/2})_c$ calculated using the value of k_{pc}.

(6) For chemicals that ionize or protonate, report the data for steps 1-5 for the experiments at the required pHs. Report the initial pH of all test chemical solutions and the type and concentration of the buffers used for each pH.

b. Actinometry Experiments

i. Treatment of Results

Follow the same discussion of the treatment of results, Section III.A.2.a.i (p. 51), to determine the rate constant of the actinometer k_{pa} using equation 2. Repeat these calculations for the trial actinometry experiments to determine the molar concentration of pyridine [PYR] needed to adjust the rate constant of the actinometer (k_{pa}) to be approximately equal to the rate constant of the test chemical (k_{pc}).

ii. Test Data Report

(1) Report the wavelength used to photolyze the actinometer.

(2) Report the size, approximate cell wall thickness, pathlength ℓ, and the type of glass used for the actinometer reaction tubes.

(3) For each trial experiment:

  (a) report the actinometer used;

  (b) report the initial concentration of actinometer $(C_o)_a$ for each replicate and the mean value;

  (c) report the molar concentration of the actinometer $(C_t)_a$ for each replicate and the mean value. Report the time t;

  (d) report the molar concentration of each replicate control sample and the mean value after the completion of the experiment. Report the time t; and

(e) report the value of k_{pa}. If small losses were observed for the actinometer, report $(k_{pa})_{obs.}$, k_{loss}, and k_{pa}.

(4) If the Moses-type PMGRR was used, list the criteria and equations used as described in Section II.J.11.c of the Technical Support Document CS-6010.

3. Phase 3 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical

a. Treatment of Results

The objective of this set of experiments is to determine the reaction quantum yield for a specific test chemical (ϕ_c) at low optical density with a low optical density actinometer. The reaction quantum yield ϕ_c can be calculated using equation 1,

$$\phi_c = (k_{pc}/k_{pa})(\varepsilon_{\lambda a}/\varepsilon_{\lambda c})\phi_a , \qquad (1)$$

by the following steps.

(1) Photolysis experiments are carried out by simultaneously photolyzing the test chemical and actinometer in the PMGRR at the chosen wavelength λ . The concentration of test chemical and actinometer are measured periodically as a function of time. These data are then used to determine the

ratio of the rate constants (k_{pc}/k_{pa}) employing linear regression analyses of the data on equation 6,

$$\ell n(C_o/C_t)_c = (k_{pc}/k_{pa})\ell n(C_o/C_t)_a \quad , \qquad (6)$$

with $\ell n(C_o/C_t)_a$ as the independent variable and $\ell n\ (C_o/C_t)_c$ as the dependent variable. The slope of the best straight line is the ratio of the rate constants (k_{pc}/k_{pa}). If a slight loss of test chemical or actinometer was detected in the controls at any time t, then employ the following procedure. Consider, as an example, the loss of test chemical in the control at time t. Using the average concentration of the test chemical in the controls from the replicates at time t and the average initial concentration, calculate $\ell n\ (C_o/C_t)_c^{loss}$. Using the average concentration of test chemical from the replicates after photolysis time t, calculate $\ell n(C_o/C_t)_c^{obs.}$. The corrected term is then

$$\ell n(C_o/C_t)_c^{corr.} = \ell n(C_o/C_t)_c^{obs.} - \ell n(C_o/C_t)_c^{loss} \quad . \qquad (27)$$

The same procedure can be applied to obtain a corrected term for the actinometer. Using the corrected terms for test chemical and/or actinometer in equation 6, determine the ratio of the rate constants (k_{pc}/k_{pa}) as described above.

(2) Determine the quantum yield of the actinometer ϕ_a using equation 7 or 8 and the molar concentration of pyridine [PYR] present in the actinometer.

(3) Use the molar absorptivities of test chemical $(\epsilon_{\lambda c})$ and actinometer $(\epsilon_{\lambda a})$ at the wavelength the photolysis experiments were carried out (i.e., 313 or 366 nm).

(4) Substitute the values of (k_{pa}/k_{pc}), $\epsilon_{\lambda a}$, $\epsilon_{\lambda c}$, and ϕ_a in equation 1 and calculate the reaction quantum yield of the test chemical ϕ_c.

A hypothetical example is presented in the Appendix, Section V., to illustrate how all the data can be used in Procedure One to determine the reaction quantum yield of the test chemical and to determine (k_{pE}) and $(t_{1/2E})$ as a function of latitude and season of the year.

b. Other Test Conditions

(1) Report the size, approximate cell wall thickness, the pathlength ℓ, and type of glass used for tubes used to hold the test chemical and actinometer solutions.

(2) Report the initial pH of all test chemical solutions, if appropriate, and the type and concentration of the buffers employed for each pH.

(3) If acetonitrile was used to solubilize the test chemical, report the percent, by volume, of the acetonitrile which was used.

(4) If significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

c. Test Data Report

(1) Report the wavelength used in the photolysis experiments.

(2) Report the actinometer used.

(3) Report the initial molar concentration of test chemical $(C_o)_c$ of each replicate and the mean value.

(4) Report the initial molar concentration of actinometer $(C_o)_a$ and the molar concentration of pyridine used.

(5) For each time point, report the two separate values for the molar concentration of test chemical $(C_t)_c$ and actinometer $(C_t)_a$ and the mean values.

(6) For each time point, report the two separate values of the molar concentration of test chemical and actinometer controls and the mean values.

(7) Tabulate and report the following data: t, $\ell n(C_o/C_t)_c$, and $\ell n(C_o/C_t)_a$. From the linear regression analysis, report the ratio of the rate constants (k_{pc}/k_{pa}), and the correlation coefficient.

(8) If loss of test chemical and/or actinometer was observed during photolysis, then report the data $\ell n(C_o/C_t)^{corr.}$, $\ell n(C_o/C_t)^{obs.}$, and $\ell n(C_o/C_t)^{loss}$ for the test chemical and/or actinometer for each time point. From the linear regression analysis of test chemical $\ell n(C_o/C_t)_c^{corr.}$ and/or actinometer $\ell n(C_o/C_t)_a^{corr.}$, report the ratio of the rate constants (k_{pc}/k_{pa}) and the correlation coefficient.

(9) Report the reaction quantum yield of the actinometer (ϕ_a).

(10) Report the reaction quantum yield of the test chemical (ϕ_c).

(11) Report $(k_{pE})_{max.}$, (k_{pE}), $(t_{1/2E})_{min.}$, and $(t_{1/2E})$ for the summer and winter solstices using the appropriate L_λ values from Tables 3-6, Test Guideline CG-6000, closest to the latitude of the chemical manufacturing site.

(12) For chemicals that ionize or protonate, report the data for steps 1-11 for the experiments at the required pHs [Section II.A.6 (p. 26)].

B. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method

1. Phase 1 Experiments: UV-Visible Absorption Spectra

The treatment of results and data reporting are exactly the same as described in Section III.A.1 (p. 47).

2. Phase 2 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical

a. Treatment of Results

The objective of this set of experiments is to determine the reaction quantum yield of a test chemical (ϕ_c) at low optical density with the high optical density ferrioxalate actinometer. ϕ_c can be calculated using equation 9,

$$\phi_c = \phi_a \, (k_{pc}/k_{pa})(2.303\varepsilon_{\lambda c}\ell)^{-1} \quad , \qquad (9)$$

by the following steps.

(1) In the first set of experiments, the ferrioxalate actinometer is photolyzed in a POB at the chosen wavelength λ (313 or 366 nm) and the molar concentration of ferrous ion $(C_t)_a$ is measured as a function of the time t. These data are fitted to equation 11 using linear regression analysis. The slope is equal to k_{pa}. The data obtained from the

third set of experiments are used in the same manner as described above to obtain another actinometer rate constant. These two rate constants are used to obtain an average actinometer rate constant $(k_{pa})_{ave.}$.

(2) In the second series of experiments, the test chemical is photolyzed in the same cell in the POB and the concentration of test chemical $(C_t)_c$ is measured as a function of the time t. These data are fitted to equation 3 using linear regression analysis. The slope is equal to k_{pc}. If a slight loss of test chemical is detected in the controls, then follow the procedure outlined in Section II.A.2.a.i (p. 19) to calculate a corrected test chemical rate constant.

(3) Use the appropriate quantum yield of the ferrioxylate actinometer (at 0.15 M) at the chosen wavelength: at 313 nm, $\phi_a = 1.20$; at 366 nm, $\phi_a = 1.18$.

(4) Use the molar absorptivity of the test chemical $(\varepsilon_{\lambda c})$ at the appropriate photolysis wavelength (313 or 366 nm) determined in Section III.B.1 (p. 60).

(5) Use the known pathlength of the reaction cell ℓ. Section II.A.3 (p. 25).

(6) Substitute the values of k_{pc}, $(k_{pa})_{ave.}$, ϕ_a, $\varepsilon_{\lambda c}$, and ℓ in equation 9 and calculate the reaction quantum yield of the test chemical ϕ_c.

(7) If the PMGRR equipment was used in these experiments, then repeat the calculations for Steps 1-6 obtained in this equipment.

b. Specific Analytical and Recovery Procedures

(1) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision; and

(2) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

c. Other Test Conditions

(1) Describe the equipment used (i.e., the type of PMGRR or POB). If a Moses-type PMGRR was used, report the aperture in cm^2.

(2) If the POB equipment was used, describe in detail the photolysis reaction cell. Report the volume, pathlength ℓ, and a description of the optical windows used.

(3) If the PMGRR equipment was used, report the size, approximate cell wall thickness, and type of glass used for the reaction tubes.

(4) If the pathlength of the cell was measured by the procedure outlined in the associated Technical Support Document, Section II.D, then report all the data obtained in determining the cell pathlength.

(5) If acetonitrile was used to solubilize the test chemical, report the percent by volume.

(6) Report the pH of all test chemical solutions, if appropriate.

(7) If a significant loss of test chemical occurred in the control samples, indicate the causes and how they were eliminated.

d. Test Data Report

(1) Report the wavelength used in the photolysis experiments.

(2) For the actinometry experiments 1 and 3;

  (i) report for each time point the two values of the molar concentration of ferrous ion formed and the mean value;

  (ii) report the actinometer rate constant k_{pa} and correlation coefficient for each experiment; and

  (iii) report the average actinometer rate constant $(k_{pa})_{ave}$.

(3) For the test chemical photolysis experiment;

- (i) report the initial molar concentration of chemical $(C_o)_c$ of each replicate and the mean value;
- (ii) report the two separate values of the molar concentration of test chemical $(C_t)_c$ and the mean value at time t. Report the time t;
- (iii) report the two separate values of the molar concentration of the test chemical controls and the mean value for each time point. Report t for each time point; and
- (iv) report from the linear regression analyses of the data, the rate constant of the test chemical (k_{pc}), and the correlation coefficient.

(4) Report the reaction quantum yield of the actinometer and (ϕ_a).

(5) Report the reaction quantum yield of the test chemical (ϕ_c).

(6) Report $(k_{pE})_{max.}$, (k_{pE}), $(t_{1/2E})_{min.}$, and $(t_{1/2E})$ for the summer and winter solstices using the appropriate L_λ values from Tables 3-6, Test Guideline CG-6000, closest to the latitude of the chemical manufacturing site.

(7) For chemicals that ionize or protonate, report the data for steps 1-6 for the experiments at the required pHs [Section II.A.6 (p. 26)].

IV. REFERENCES

Andre JC, Niclause M, Joussat-Dubien J, and Deglise X. 1977. Photodegradation of pyridine in aqueous solution. J Chem Ed 54:387.

ASTM. 1983. Annual Book of ASTM Standards, American Society for Testing and Materials, Philadelphia, PA. Part 31, Method D 1193-77.

Calvert JG and Pitts JN. 1966. Photochemistry. John Wiley and Sons, Inc., New York, New York.

de Mayo P and Shizuka P. 1976. Measurement of reaction quantum yields. "Creation and Detection of the Excited State," Vol. 4, A.R. Ware, Ed. Marcel Dekker, Inc., New York, New York.

Dulin D and Mill T. 1982. Development and application of solar actinometers. Environ Sci and Tech 16:815.

Handbook of Chemistry and Physics. 1983. The Chemical Rubber Co., Cleveland, Ohio.

Hatchard CG and Parker CA. 1956. A new sensitive chemical actinometer II. Potassium ferrioxylate as a standard chemical actinometer. Proc Royal Soc of London A 235:518.

Mill T, Davenport JE, Dulin DE, Mabey WR, and Bawol R. 1981. Evaluation and optimization of photolysis screening protocols. EPA-560/5-81-003.

Mill T, Mabey WR, Hendry DG, Winterle J, Davenport JE, Barich V, Dulin D, and Tse D. 1982. Design and validation of screening and detailed methods for environmental processes. EPA- .

Mill T, Mabey WR, Bomberger DC, Chou T-W, Hendry DG, and Smith JH. 1982a. Laboratory protocols for evaluating the fate of organic chemicals in air and water. EPA-600/3-82-022.

Murov SL. 1973. Handbook of photochemistry. Marcel Dekker, Inc., New York, New York.

Moses FG, Liu RSH, and Monroe BM. 1969. The "merry-go-round" quantum yield apparatus. Mol Photochem 1:245.

Parker CA. 1953. A new sensitive chemical actinometer I. Some details with potassium ferrioxylate. Proc Ray Soc of London A220:104.

Zepp RG. 1978. Quantum yields for reaction of pollutants in dilute aqueous solution. Environ Sci and Technol 12:327.

V. APPENDIX

Hypothetical Illustrative Example: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method and Sunlight Photolysis

Consider a chemical plant just south of Peoria, Illinois on the Illinois River which produces an organic chemical A which is not an acid or a base. The waste effluent passes through a primary and secondary treatment plant and the waste, which still contains some chemical A, is then discharged into the river. The plant is located at 40.7 degrees north latitude. Information is needed on photolysis rates and half-lives of chemical A in aqueous media in the summer and winter seasons.

The company reseach laboratory is located in Peoria, Illinois and the required photolysis data was needed in January. Since the temperature outdoors was well below freezing during this month, the outdoor sunlight photolysis experiments, described in Test Guideline CG-6000, could not be carried out. Thus, it was necessary to carry out photolysis experiments in the laboratory. The research laboratory was equipped to carry out photolysis experiments with an Ace-type PMGRR and thus Procedure One was used.

Laboratory Experiments, Data, and Calculations

1. Phase 1 Experiments

Chemical A had a saturated water solubility of 3.9×10^{-3} M at 25°C. In the Phase 1 procedure, the uv-visible absorption spectrum was obtained for chemical A at a concentration of 1.00×10^{-5} M in a 10.0 cm quartz cell in duplicate. Using the wavelength interval range from Table 1 of Test Guideline CG-6000, the average absorbance of duplicate runs at λ center was obtained and the results are summarized in Table 1.* In addition, the average absorbance was measured at 313.0 nm and at 366.0 nm and these results are summarized in Table 1. From the above data and equation 25, the average molar absorptivity is

$$\varepsilon_{\lambda c} = 10^4 A_{\lambda c} \quad . \tag{28}$$

---

*It must be emphasized that $\varepsilon_{\lambda c}$ has to be averaged over the wavelength intervals that correspond to the same intervals for the L_λ values centered at λ . This has already been taken care of in this Test Guideline since the wavelength interval ranges listed in Table 1 coincide with the same wavelength intervals for L_λ centered at λ in Tables 3-6 of Test Guideline CG-6000.

TABLE 1. SUMMARY OF SPECTRAL AND PHOTOLYSIS DATA FOR CHEMICAL A

A. SPECTRAL DATA

λ center (nm)	$A_{\lambda c}$	$\epsilon_{\lambda c}(M^{-1}cm^{-1})$
297.5	0.5904	5904
300.0	0.5414	5414
302.5	0.4924	4924
305.0	0.4434	4434
307.5	0.3810	3810
310.0	0.3124	3124
312.5	0.2714	2714
315.0	0.2224	2224
317.5	0.2004	2004
320.0	0.1514	1514
323.1	0.1310	1310
330.1	0.1030	1030
340.0	0.0645	645
350.0	0.0473	473
360.0	0.0287	287
370.0	0.0170	170
380.0	0.0085	85
390.0	0.0030	30
400.0	0.0010	10
410.0	0.0000	0
313.0	0.2610	2610
366.0	0.0185	185

TABLE 2. Continued

B. PHOTOLYSIS DATA

	Summer		Winter	
λ center (nm)	L_λ^*	$\epsilon_\lambda c L_\lambda$ (day^{-1})	L_λ^*	$\epsilon_\lambda c L_\lambda$ (day^{-1})
297.5	6.17(-5)	0.4	5.49(-7)	0.0
300.0	2.70(-4)	1.5	5.13(-6)	0.0
302.5	8.30(-4)	4.1	3.02(-5)	0.2
305.0	1.95(-3)	8.7	1.19(-4)	0.5
307.5	3.74(-3)	14.3	3.38(-4)	1.3
310.0	6.17(-3)	19.3	7.53(-4)	2.4
312.5	9.07(-3)	24.6	1.39(-3)	3.8
315.0	1.22(-2)	27.1	2.22(-3)	4.9
317.5	1.55(-2)	31.1	3.19(-3)	6.4
320.0	1.87(-2)	28.3	4.23(-3)	6.4
323.1	3.35(-2)	43.9	8.25(-3)	10.8
330.0	1.16(-1)	119.5	3.16(-2)	32.6
340.0	1.46(-1)	94.2	4.31(-2)	27.8
350.0	1.62(-1)	76.6	4.98(-2)	23.6
360.0	1.79(-1)	51.4	5.68(-2)	16.3
370.0	1.91(-1)	32.5	6.22(-2)	10.6
380.0	2.04(-1)	17.3	6.78(-2)	5.8
390.0	1.93(-1)	5.8	6.33(-2)	1.9
400.0	2.76(-1)	2.8	9.11(-2)	0.9
410.0	3.64(-1)	0.0	1.20(-1)	0.0
		$\sum \epsilon_\lambda c L_\lambda$ = 603.4		$\sum \epsilon_\lambda c L_\lambda$ =156.2

---

*The units of L_λ are in 10^{-3} einsteins cm^{-2} day^{-1}. The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

From the average value of $A_{\lambda c}$ at λ center, the average molar absorptivity can be obtained from equation 28 and these results are summarized in Table 1.A. In addition, using $A_{\lambda c}$ at 313.0 and 366.0 nm in equation 28, the molar absorptivity ε_{313c} and ε_{366c} can be obtained and these values are also given in Table 1.A.

Since the plant is located at 40.7 degrees north latitude, the closest L_λ values are at 40 degrees north latitude. These values are obtained from Table 5 of Test Guideline CG-6000 and are summarized in Table 1.B. for the summer and winter solstices. Using the data from Table 1.B. and equations 16 and 17, the following results are obtained.

Summer Solstice	Winter Solstice
$(k_{pE})_{max.} = \sum \varepsilon_{\lambda c} L_\lambda = 603 \text{ days}^{-1}$	$(k_{pE})_{max.} = \sum \varepsilon_{\lambda c} L_\lambda = 156 \text{ days}^{-1}$
$(t_{1/2E})_{min.} = 1.1 \times 10^{-3}$ days	$(t_{1/2E})_{min.} = 4.4 \times 10^{-3}$ days

Since the chemical transforms rapidly on the summer and winter solstices, it is necessary to determine the reaction quantum yield of chemical A in the laboratory using Procedure One and to obtain direct sunlight photolysis rates and half-lives in aqueous media during the summer and winter seasons.

2. Phase 2 Experiments

a. Determination of the Approximate Rate Constant of the Test Chemical

Chemical A was dissolved directly in pure water and a homogeneous solution was prepared. Analysis of duplicate samples indicated that the average concentration was 5.00×10^{-6} M. Using the uv spectral data obtained, the absorbance at 313 nm in a one cm absorption cell containing a solution at a concentration of 5.00×10^{-6} M was less than 0.02 [i.e., $A_{313c} = 2610\ (5.00 \times 10^{-6})(1.00) = 0.0131$]. Hence, the test chemical solution in approximately 1 cm pathlength tubes was at low optical density.

A series of tubes (13 x 100 mm), with an effective pathlength of 1.12 cm, were filled with the aqueous solution of test chemical and sealed. Since $\varepsilon_{313c} > \varepsilon_{366c}$ (Table 1), photolysis experiments were carried out at 313 nm in a PMGRR using the procedure described in Section II.B.2.a (p. 37). Duplicate photolyzed and control samples were removed periodically and analyzed for the concentration of test chemical. At t = 39.4 hours, the average concentration of photolyzed sample $(C_t)_c$ was 2.25×10^{-6} M and the average concentration of control sample was 4.99×10^{-6} M. Thus, approximately 55% of the test chemical transformed. Since no loss of chemical was observed in the control sample, no adventitious processes occurred and the loss of chemical was only due to photolysis. Using equation 3 and the above data:

$$\ln(5.00 \times 10^{-6}/2.25 \times 10^{-6}) = k_{pc}(39.4)$$

$$k_{pc} = 0.0203 \text{ hours}^{-1} . \qquad (29)$$

b. Actinometry Experiments

Utilizing equation 5 and the value of k_{pc} obtained in equation 29, the half-life of the test chemical is then

$$(t_{1/2})_C = 0.693/0.0203 = 34.1 \text{ hours}. \qquad (30)$$

Since $(t_{1/2})_C > 12$ hours, the PNAP/PYR actinometer is required for the photolysis experiments.

Using equation 18 and the approximate rate constant k_{pc} from equation 29, the concentration of pyridine needed to make the rate constant of the actinometer approximately equal to the rate constant of the test chemical is given below:

$$[PYR] = 1.16\ k_{pc} = 1.16(0.0203)$$

$$[PYR] = 2.36 \times 10^{-2}\ M \quad . \qquad (31)$$

An actinometer solution was then prepared according to the procedure given in Section II.B.2.b.i (p. 40) and the concentration of pyridine was 2.36×10^{-2} M. The concentration of PNAP was measured in duplicate and the average concentration (C_o) was 0.900×10^{-5} M. Using uv spectral data, the absorbance at 313 nm in a one cm absorption cell containing an actinometer solution at a concentration of 0.900×10^{-5} M was less than 0.02

(i.e., $A_{313a} = (2056)(0.900 \times 10^{-5})(1.00) = 0.0185$]. Hence, the actinometer solution in approximately 1 cm pathlength tubes was at low optical density.

A series of tubes, identical to those used in the test chemical photolysis experiments, were filled with the actinometer solution and sealed. The photolysis experiments were carried out in the same PMGRR at 313 nm using the procedure described in Section II.B.2.b.ii (p. 42). Duplicate photolyzed and control samples were periodically removed and analyzed for the concentration of PNAP. At 50.6 hours, the average concentration of photolyzed sample $(C_t)_a$ was 0.360×10^{-5} M and the average concentration of the control sample was 0.900×10^{-5} M. Thus, approximately 60% of PNAP transformed. Since no loss of chemical was observed in the control sample, no adventitious processes occured and the loss of chemical was only due to photolysis. Using equation 2 and the above data:

$$\ln(0.900 \times 10^{-5}/0.360 \times 10^{-5}) = k_{pa}\ (50.6)$$

$$k_{pa} = 0.0181 \text{ hours}^{-1} \quad . \qquad (32)$$

Thus, at the molar concentration of pyridine given by equation 31, k_{pa} is approximately the same as k_{pc} (within ± 50%).

3. Phase 3 Experiments

A solution of test chemical was prepared according to the procedure described in Section II.B.2.a.ii (p. 38) and the concentration $(C_o)_c$ was measured in duplicate and the average concentration was found to be 5.00 x 10^{-6} M. An actinometer solution (PNAP/PYR) was prepared according to the procedure described in Section II.B.2.b.i (p. 40) with a pyridine concentration of 2.36 x 10^{-2} M (equation 31). The average concentration of PNAP $(C_o)_a$ from duplicate samples was measured and found to be 9.00 x 10^{-6} M. These solutions were placed in identical tubes (13 x 100 mm), sealed, and photolyzed at 313 nm in the PMGRR according to the procedure given in Section II.B.3 (p. 43). The average concentration of duplicate samples of test chemical, test chemical control, actinometer (PNAP), and actinometer control, obtained in this photolysis experiment, is summarized in Table 2.

Table 2. Photolysis Data for Test Chemical A and (PNAP/PYR) Actinometer

	Average Test Chemical Concentration x10^6		Average PNAP Concentration x 10^6	
t(hours)	Photolyzed Samples	Controls	Photolyzed Samples	Controls
0	5.00	5.00	9.00	9.00
12	3.92	4.98	7.24	8.99
24	3.07	5.00	5.83	8.98
36	2.41	4.99	4.69	9.00
48	1.89	4.98	3.78	8.98
60	1.48	4.99	3.04	8.99
72	1.16	5.00	2.44	9.00

Since no significant loss of PNAP or test chemical was observed in the control samples, no adventitious processes occurred and the loss of test chemical and PNAP was only due to photolysis. Utilizing the data in Table 2, $\ln(C_o/C_t)$ for the test chemical and actinometer solution can be calculated and the results are summarized in Table 3.

Table 3. Photolysis Data for Test Chemical and Actinometer (PNAP/PYR)

	Test Chemical		Actinometer (PNAP)	
t (hours)	$(C_t)_c \times 10^6$(M)	$\ln(C_o/C_t)_c$	$(C_t)_a \times 10^6$(M)	$\ln(C_o/C_t)_a$
0	5.00	0.000	9.00	0.000
12	3.92	0.243	7.24	0.218
24	3.07	0.488	5.83	0.434
36	2.41	0.730	4.69	0.652
48	1.89	0.973	3.78	0.868
60	1.48	1.22	3.04	1.09
72	1.16	1.46	2.44	1.31

The ratio of the rate constants (k_{pc}/k_{pa}) is defined by equation 6,

$$\ln(C_o/C_t)_c = (k_{pc}/k_{pa}) \ln(C_o/C_t)_a \quad . \qquad (6)$$

Utilizing all the data in Table 3 including the time point $t = 0$ and linear regression analysis, the slope is found to be 1.12 with a correlation coefficient of 1.000.

The quantum yield of the PNAP/PYR actinometer is given by equation 7,

$$\phi_a = 0.0169\ [PYR]. \tag{7}$$

Since the pyridine concentration in the actinometer was 2.36×10^{-2} M (equation 31), the quantum yield of the actinometer (ϕ_a) is 3.99×10^{-4}.

The reaction quantum yield of the test chemical is given by equation 1,

$$\phi_c = (k_{pc}/k_{pa})(\varepsilon_{\lambda a}/\varepsilon_{\lambda c})\ \phi_a \quad . \tag{1}$$

The pertinent data are summarized as follows:
$\phi_a = 3.99 \times 10^{-4}$, $(k_{pc}/k_{pa}) = 1.12$, $\varepsilon_{\lambda c} = 2610$, and $\varepsilon_{\lambda a} = 2056$. Substituting these data in equation 1 yields

$$\phi_c = (1.12)(2056/2610)(3.99 \times 10^{-4})$$

$$\phi_c = 3.52 \times 10^{-4} \quad . \tag{33}$$

The rate constants for direct photolysis of test chemical in aqueous media and the half-life for water bodies and clear sky conditions for the winter and summer seasons can be calculated as follows: The values of $\sum \varepsilon_{\lambda c} L_{\lambda}$ have been calculated for the summer and winter solstices, Table 1.B. For summer $\sum \varepsilon_{\lambda c} L_{\lambda} = 603 \text{ days}^{-1}$; for winter $\sum \varepsilon_{\lambda c} L_{\lambda} = 156 \text{ days}^{-1}$. The reaction quantum yield for chemical A is 3.52×10^{-4} (equation 33). Using these data in equation 16 yields

$$\underline{\text{summer}}\text{:}\quad k_{pE} = 3.52 \times 10^{-4}(603) = 0.213 \text{ days}^{-1} \tag{34}$$

$$\underline{\text{winter}}\text{:}\quad k_{pE} = 3.52 \times 10^{-4}(156) = 0.0549 \text{ days}^{-1}. \tag{35}$$

These values can be substituted into equation 18 to obtain the half-lives for these two seasons.

$$\underline{\text{summer}}\ (t_{1/2E})_c = 0.693/0.213 = 3.3 \text{ days} \tag{36}$$

$$\underline{\text{winter}}\ (t_{1/2E})_c = 0.693/0.0549 = 13 \text{ days} \quad . \tag{37}$$

These results are valid for clear-sky conditions and at shallow depths in the Illinois River.

CS-6010
October, 1984

LABORATORY DETERMINATION OF THE DIRECT PHOTOLYSIS REACTION QUANTUM YIELD IN AQUEOUS SOLUTION AND SUNLIGHT PHOTOLYSIS

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Contents

Page

I. NEED FOR THE TEST.. 1

II. SCIENTIFIC ASPECTS.. 4

- A. Historical Discussion....................................... 4
- B. Theoretical Discussion...................................... 9
- C. Actinometry... 14
  - 1. Low Optical Density Actinometers.................... 16
    - a. p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR).. 16
    - b. p-Nitroanisole-Pyridine Actinometer (PNA/PYR)... 17
  - 2. High Optical Density Actinometers................... 18
    - a. Potassium Ferrioxalate Actinometer............. 19
    - b. o-Nitrobenzaldehyde Actinometer................. 23
    - c. Reinecke's Salt Actinometer..................... 25
- D. Determination of the Cell Pathlength.................... 26
- E. Development of the Test Method.......................... 30
  - 1. Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method..................... 32
    - a. Theoretical Discussion........................... 32
    - b. Procedure One: Determination of the Reaction Quantum Yield by the Simultaneous Photolysis of Test Chemical and Actinometer at Low Optical Density.......................... 34
      - i. Phase 1 Experiments.................... 35
      - ii. Phase 2 Experiments.................... 36
      - iii. Phase 3 Experiments.................... 37
  - 2. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method.. 38
    - a. Theoretical Discussion........................... 38
    - b. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method............. 39

Conts. (Continued)

Page

- i. Phase 1 Experiments........................ 40
- ii. Phase 2 Experiments........................ 40

3. Procedure Three: Determination of the Reaction Quantum Yield by the High Optical Density Test Chemical and Actinometer Method........ 42

F. Quantum Yield as a Function of Wavelength............... 46
G. Photolysis and Molar Absorptivity in Water.............. 46
H. Solar Irradiance Data................................... 47
I. Applicability and Specificity of the Test Method........ 48
J. Rationale for the Selection of the Test Conditions...... 50

1. UV-Visible Absorption Spectrophotometer............. 50
2. Special Photochemical Laboratory Equipment.......... 50
    - a. Design of Apparatus.............................. 50
    - b. Light Sources.................................... 51
    - c. Light Filtering Systems.......................... 52
    - d. Reaction Cells................................... 54
    - e. Temperature Control.............................. 55

3. Cell Pathlength..................................... 55
4. Solvents.. 56
5. Sterilization....................................... 57
6. Preparation of Test Chemical Solution............... 58
7. PH Effects.. 58
8. Volatile Chemical Substances........................ 59
9. Control Solution.................................... 60
10. Absorption Spectrum as a Criterion for Performing the Reaction Quantum Yield Experiments.................................. 61
11. Actinometry... 61
    - a. p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR) in an Ace-Type PMGRR................ 63
    - b. p-Nitroanisole-Pyridine Actinometer (PNA/PYR) in an Ace-Type PMGRR.................. 65
    - c. The PNAP/PYR and PNA/PYR Actinometers in a Moses-Type PMGRR......................... 67

12. Chemical Analysis of Solutions...................... 71
    - a. Chemical Analysis of Test Chemical Solutions.... 71
    - b. Chemical Analysis of p-Nitroacetophenone (PNAP) 72
    - c. Chemical Analysis of p-Nitroanisole (PNA)....... 72
    - d. Chemical Analysis of Ferrous Ion in the Ferrioxalate Actinometer....................... 73

Conts. (Continued)

Page
III. REFERENCES.. 74
IV. APPENDIX: GLOSSARY OF IMPORTANT SYMBOLS................ 77

236

LABORATORY DETERMINATION OF THE DIRECT PHOTOLYSIS REACTION QUANTUM YIELD IN AQUEOUS SOLUTION AND SUNLIGHT PHOTOLYSIS

I. NEED FOR THE TEST

The majority of the earth's surface is covered by water in the form of oceans, seas, rivers, lakes, streams, or ponds. As a result, numerous chemicals enter natural aquatic systems from a variety of sources. For example, chemical wastes are discharged directly into natural water bodies, and chemicals leach into natural water bodies from landfills. Pesticides are applied directly into water bodies, and are applied to soils and vegetation and subsequently migrate into water bodies. Some pollutants present in aqueous media can undergo photochemical transformation in the environment (i.e., in sunlight by direct photolysis or by sensitized photolysis). As a result, there is considerable interest in photolysis in solution, especially the photolysis of pesticides. However, most of these studies have been qualitative in nature and involved the identification of photolysis products. Quantitative data in the form of rate constants and half-lives are needed to determine the importance of photochemical transformation of pollutants in aqueous media.

Direct aqueous photolysis represents the transformation of a chemical substance by direct absorption of radiant energy (sunlight) into products different from their precursors. Chemical substances which are present in aqueous media photolyze at different rates depending upon the solar irradiance, the chemical substance's molar absorptivity at each wavelength of solar radiation, and its photolysis reaction quantum yield at the wavelengths of concern. Chemical substances which photolyze rapidly under environmental conditions have relatively short lifetimes in the environment. Consequently, the chemical fate assessment may focus on the transformation products to a greater extent than on the parent compound. On the other hand, if the chemical substance is resistant to photolysis as well as to all the other possible transformation processes, the assessment should focus on the parent chemical.

Test Guideline CG-6000, the first in a series of aqueous photolysis tests, was designed to determine the molar absorptivity and the reaction quantum yield of a test chemical in aqueous solution in sunlight. These parameters are then combined with solar irradiance data to determine environmental rate constants in aqueous solution as a function of latitude and season of the year anywhere in the United States.

Test Guideline CG-6000 was developed as a screening test to obtain the direct photolysis reaction quantum yield of a chemical in aqueous solution by carrying out photolysis experiments in sunlight. This method does not require sophisticated and expensive photochemical equipment and therefore the reaction quantum yield can be easily determined and at a moderate cost. However, there are circumstances when this method may not be applicable. For example, this procedure is not applicable when the temperature outdoors falls below zero degrees centigrade. Furthermore, depending upon the status of a risk assessment for a specific chemical, a more precise value of the reaction quantum yield may be required. Thus, a more comprehensive procedure is needed to determine the reaction quantum yield in the laboratory using specialized photochemical equipment and monochromatic light.

Test Guideline CG-6010, the second guideline in a series of photolysis procedures, represents an upper-tier test to determine the reaction quantum yield in the laboratory. These data will be used to determine how rapidly direct photolysis will take place in aqueous media under certain environmental conditions. If the photolysis data indicate that photolysis is a relatively important transformation process and that the initial risk assessment indicates that there is a threat to the health of humans and/or the environment, then other upper-tier photolysis tests may be required to obtain more precise photolysis data over a wide range of environmental conditions. These upper-tier tests will also be concerned with determining the identity and fate of the transformation products.

II. SCIENTIFIC ASPECTS

A. Historical Discussion

An historical discussion of the theory and methods of determining direct photolysis rate constants and half-lives in the environment (i.e., in sunlight) is given in detail in Support Document CS-6000, Photolysis in Aqueous Solution in Sunlight. In addition, Zepp (1982) gives a detailed discussion of experimental approaches to environmental photochemistry. The following paragraphs give a brief summary of some of the relevant principles of environmental photochemistry.

Zepp and Cline (1977) published a paper describing the theory of calculating rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water body. It has been shown that in an optically thin aqueous solution (i.e., the absorbance of a chemical is less than 0.02 in the reaction cell at all wavelengths greater than 290 nm), the kinetic expression for direct photolysis of a chemical at a molar concentration C is given by the first-order differential equation

$$-\,(dC/dt) = \phi_E k_a C = k_{pE} C \qquad (1)$$

$$k_{pE} = \phi_E k_a \quad , \qquad (2)$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength (Section II.F), and $k_a = \sum k_{a\lambda}$, the sum of $k_{a\lambda}$ values for all wavelengths of sunlight that are absorbed by the chemical (i.e., the light absorption rate constant). The term k_{pE} represents the first-order direct photolysis rate constant for a chemical in a water body in sunlight in the units of reciprocal time. Integrating equation 1 yields

$$\ell n(C_o/C_t) = k_{pE}t \quad , \qquad (3)$$

where C_t is the molar concentration of chemical at time t during photolysis and C_o is the initial molar concentration. By measuring the concentration of chemical as a function of the time t during photolysis in sunlight, k_{pE} can be determined using equation 3. In addition, equation (3) can be solved for the condition $C_t = C_o/2$ and the half-life of the chemical is given by

$$t_{1/2E} = 0.693/k_{pE} \quad . \qquad (4)$$

Furthermore, under the same conditions cited above [i.e., for a homogeneous chemical solution with absorbance less than 0.02 in a reaction cell at all wavelengths greater than 290 nm and at shallow depths (less than 0.5 m)], the first-order direct photolysis rate constant, k_{pE}, is

$$k_{pE} = \phi_E \sum \varepsilon_{\lambda c} L_\lambda \quad , \qquad (5)$$

where ϕ_E is the reaction quantum yield which is independent of the wavelength, $\varepsilon_{\lambda c}$ is the molar absorptivity in the units molar^{-1} cm^{-1}, and L_λ is the solar irradiance in water in the units 10^{-3} einsteins cm^{-2} day^{-1} [Mill et al. (1981, 1982a, 1982b)]. L_λ is the day averaged solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

A simple screening test has been developed in Test Guideline CG-6000 using equation 5. As an approximation, it has been assumed that the reaction quantum yield ϕ_E is equal to one, the maximum value.* As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 5 becomes

$$(k_{pE})_{max.} = \sum \varepsilon_{\lambda c} L_\lambda \quad . \qquad (6)$$

Substituting equation 6 in equation 4, the lower limit for the half-life is then given by

---

*It is possible that under certain circumstances the reaction quantum yield can be greater than 1. For example, this happens when a chain reaction occurs after the absorption of a quantum of light. However, this rarely occurs in dilute aqueous solution.

$$(t_{1/2E})_{\text{min.}} = 0.693/(k_{pE})_{\text{max.}} \quad . \tag{7}$$

In order to estimate $(k_{pE})_{\text{max.}}$ using equation 6, it is necessary to obtain the molar absorptivities $(\varepsilon_{\lambda c})$ of the test chemical and the solar irradiance data L_λ . Details for determining $\varepsilon_{\lambda c}$ are given in Test Guideline CG-6000 along with a listing of solar irradiance data L_λ (Tables 3-6). A procedure is described how to use these data to estimate $(k_{pE})_{\text{max.}}$ and $(t_{1/2E})_{\text{min.}}$ using equations 6 and 7.

Test Guideline CG-6000 also describes a procedure for determining the reaction quantum yield of a test chemical in aqueous solution in sunlight. The reaction quantum yield can then be combined with the $\varepsilon_{\lambda c}$ and L_λ data in equation 5 to determine the direct photolysis rate constant k_{pE} and the corresponding half-life $t_{1/2E}$ using equation 4. Furthermore, it should be noted that the method is very general and can be extended to determine rates of photolysis over a range of environmental conditions using a computer program. Zepp and Cline (1977) have written a computer program to calculate rates of photolysis as a function of depth in water, in natural waters as a function of the water absorption or attenuation coefficient (α_λ) , the average ozone layer that pertains to the season and location of interest, and as a function of latitude and season of the year. This program has been recently updated with the best available solar irradiance data and is called the GC SOLAR computer program. The GC SOLAR computer program is available on request [R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601].

Test Guideline CG-6000 was developed as a screening test to determine the reaction quantum yield of a test chemical in aqueous solution by carrying out photolysis experiments in sunlight. This method does not require sophisticated and expensive photochemical equipment and thus the reaction quantum yield can be determined easily and at a modest cost. However, the method does have some limitations. For example, this Test Guideline can only be carried out during the warm seasons of the year in the United States. Unfortunately, in many parts of this country, the temperature falls below 0°C during the winter months. Therefore, if photolysis experiments are carried out in the winter in certain parts of the country, the dilute aqueous solutions would freeze, the tubes would break, and the samples would be destroyed. Furthermore, under certain circumstances, a more precise value of the reaction quantum yield may be required. For example, if the photolysis test data indicate that photolysis is a relatively important transformation process and the initial risk assessment indicates that there is a threat to the health of humans and/or the environment, then an upper-tier test may be required to obtain a more precise value of the reaction quantum yield for a specific test chemical. Finally, some laboratories have photochemical equipment using monochromatic light already set up and may prefer to use it.

Thus, a more comprehensive procedure is needed to determine the reaction quantum yield of a test chemical in the laboratory using specialized photochemical equipment.

B. Theoretical Discussion

Methods for determining the reaction quantum yield* of pollutants in dilute aqueous solution have been described by Zepp (1978) and some theoretical aspects of photochemistry will be discussed in this section.

Based on the first and second laws of photochemistry, the general rate equation for aqueous photolysis of a chemical in a cell containing volume V of solution and exposed area A is

$$\text{Rate} = -(dC/dt)_{\lambda} = \phi I_{O\lambda}(A/V)F_{S\lambda}\ F_{C\lambda} \quad , \qquad (8)$$

where C is the molar concentration of the chemical, t is the time, ϕ is the reaction quantum yield of the chemical, $I_{O\lambda}$ is the incident light intensity at wavelength λ, $F_{S\lambda}$ is the fraction of the light absorbed by the system, and $F_{C\lambda}$ is the fraction of light absorbed by the chemical [Balzani and Carassiti (1970]. For complex organic molecules in solution, ϕ is assumed to be independent of the wavelength, Section II.F. The terms $F_{s\lambda}$ and $F_{c\lambda}$ are defined by the equations

---

*Since the term quantum yield is applied to other photochemical processes including fluorescence, phosphorescence, and sensitizations, the term "reaction quantum yield" should always be explicitly stated.

$$F_{s\lambda} = 1 - 10^{-(\alpha_\lambda + \varepsilon_\lambda C)\ell} \qquad (9)$$

$$F_{c\lambda} = \varepsilon_\lambda C/(\alpha_\lambda + \varepsilon_\lambda C) \quad , \qquad (10)$$

where α_λ is the absorption (or attenuation) coefficient of water, ε_λ is the molar absorptivity of the chemical, and ℓ is the pathlength of light.

By letting $I_\lambda = I_{o\lambda}(A/V)$, equation 8 becomes

$$\text{Rate} = -(dC/dt)_\lambda = \phi I_\lambda F_{s\lambda} F_{c\lambda} \ . \qquad (11)$$

For an aqueous solution of a chemical with low optical density or absorbance, the incident light on the solution is weakly absorbed. That is, only a fraction of the incident light is absorbed by the system. $F_{s\lambda}$, defined by equation 9, takes the form

$$F_{s\lambda} = 1 - 10^{-(\alpha_\lambda + \varepsilon_\lambda C)\ell} = 1 - \exp[-2.303(\alpha_\lambda + \varepsilon_\lambda C)\ell] \qquad (11a)$$

$$F_{s\lambda} = 1 - \exp(-x) \quad , \qquad (12)$$

where $$x = 2.303\,(\alpha_\lambda + \varepsilon_\lambda C)\ell \quad ; \qquad (13)$$

and the expansion of exp(-x) in a series [Pierce (1929)] yields

$$\exp(-x) = 1 - x + x^2/2! - x^3/3! + \quad .... \qquad . \qquad (14)$$

For weak absorbance, $x < 0.02$; and thus the terms $x^2/2!$, $x^3/3!$, . . . are small relative to x and can be neglected. Thus, equation 14 becomes

$$x = 1 - \exp(-x) \qquad . \qquad (15)$$

Substituting equation 15 into equation 12 and utilizing equation 13 yields

$$F_{s\lambda} = x = 2.303(\alpha_\lambda + \varepsilon_\lambda C)\ell \qquad . \qquad (16)$$

Substituting equations 16 and 10 in equation 11 yields the first-order differential equation

$$-(dC/dt)_\lambda = 2.303\phi I_\lambda \varepsilon_\lambda \ell C$$

$$-(dC/dt)_\lambda = k_p C \qquad , \qquad (17)$$

where k_p is the first-order rate constant and is given by the expression

$$k_p = 2.303\phi I_\lambda \varepsilon_\lambda \ell \qquad . \qquad (18)$$

Integrating equation 17 under boundary conditions $(t = 0, C_o)$ and (t, C_t) yields

$$\ell n(C_o/C_t) = k_p t \quad . \qquad (19)$$

Equation 19 can be solved for the condition $C_t = C_o/2$ and the half-life of the chemical $(t_{1/2})$ is given by

$$t_{1/2} = 0.693/k_p \quad . \qquad (20)$$

For an aqueous solution of a chemical (e.g., an actinometer or test chemical) with high optical density or absorbance, essentially all the light is absorbed. Under these conditions the absorbance is >2. Since the absorbance is equal to $(\alpha_\lambda + \varepsilon_\lambda C)\ell$ and is >2, the term

$$10^{-(\alpha_\lambda + \varepsilon_\lambda C)\ell} \ll 1 \quad ,$$

and $F_{s\lambda}$ defined by equation 9 is then given by the relationship

$$F_{s\lambda} = 1 \quad . \qquad (21)$$

Furthermore, since essentially all the light is absorbed by the chemical, $\varepsilon_\lambda C \gg \alpha_\lambda$ and $F_{c\lambda}$ defined by equation 10 is then given by the relationship

$$F_{c\lambda} = 1 \quad . \qquad (22)$$

Substituting the results of equations 21 and 22 in equation 11 yields the zero-order differential equation

$$-(dC/dt) = \phi I_{\lambda} \quad . \qquad (23)$$

Integrating equation 23 under the boundary conditions $(t=0, C_o)$ and (t, C_t) yields

$$(C_o - C_t) = k_p t \quad , \qquad (24)$$

where k_p is the zero-order rate constant and is given by the expression

$$k_p = \phi I_{\lambda} \quad . \qquad (25)$$

Equations 23, 24, and 25 are applicable to the disappearance of chemical (e.g., test chemical and actinometer) and the reaction kinetics are studied by measuring the disappearance of chemical as a function of time. However, there are instances when the reaction kinetics are studied by following the formation of one of the products (e.g., the ferrous ion in the ferrioxalate actinometer discussed in Section II.C.2.a). For a condition in which the rate of disappearance of chemical equals the rate of formation of one of the products,

$$-(dC_r/dt)_\lambda = (dC_p/dt)_\lambda \quad , \qquad (26)$$

where C_r is the molar concentration of reactant and C_p is the molar concentration of one of the products. Using equation (26) in equation 11 and proceeding in the same manner as above, the following equations are obtained:

$$(dC_p/dt) = \phi I_\lambda \qquad (27)$$

$$(C_t)_p = k_p t \qquad (28)$$

$$k_p = \phi I_\lambda \quad , \qquad (29)$$

where $(C_t)_p$ now represents the molar concentration of the product formed at time t.

C. Actinometry

Chemical actinometers are used in reaction quantum yield experiments to measure the integrated light intensity incident on the sample during photolysis. Chemical actinometers are photochemical reactions which have been calibrated directly or indirectly with light sources of known light flux and have well defined reaction quantum yields ϕ_a at specific wavelengths λ .

In the environment, photolysis takes place in aqueous media. Thus, the reaction quantum yield of a test chemical is required in aqueous solution. Complications are encountered when reaction quantum yield experiments are carried out in the recommended cylindrical cells (Section II.D) with water as the solvent for the test chemical and the actinometer is dissolved in an organic solvent. These complications arise because the light flux measured is a function of the refractive index of the water and the organic solvent relative to the glass used in the fabrication of the cylindrical cells. As a result, the reaction quantum yield measured can be in error. This effect will be discussed in more detail in Section II.D. Therefore, in order to measure precise reaction quantum yields of test chemicals in cylindrical cells, actinometers in aqueous solution are highly desirable. Four aqueous actinometers will be described in the following sections which can be used to obtain environmentally relevant reaction quantum yields in the spectral region 290-750 nm. In cases where aqueous solutions are not practical, the recommended actinometer solvent is acetonitrile. Acetonitrile has an index of refraction $n^{20°} = 1.344$ which is close to the refractive index of water ($n^{20°} = 1.332$). Thus, the o-nitrobenzaldehyde actinometer dissolved in acetonitrile is also described (Section II.C.2.b) and can be used in these reaction quantum yield experiments. There are other actinometers that could be used in these experiments. However, if actinometers other than the ones described in this section are used, they must be completely described. For detailed information on actinometers, the descriptions by Calvert and Pitts (1966), de Mayo and Shizuka (1976), and Murov (1973) are highly recommended.

1. Low Optical Density Actinometers

Two low optical density actinometers will be described which can be used to determine the reaction quantum yield of a test chemical at low optical density (i.e., absorbance < 0.02). These actinometers are ideal for environmental actinometry experiments since they are in aqueous solution and have variable reaction quantum yields which are well defined. These two low optical density actinometers, composed of the systems p-nitroacetophenone-pyridine and p-nitroanisole-pyridine, have been recently developed for the Office of Toxic Substances, U.S. Environmental Protection Agency, by SRI International [Mill et al. (1981, 1982) and Dulin and Mill (1982)]. The p-nitroacetophenone-pyridine actinometer has already been incorporated in Test Guideline CG-6000 to measure the reaction quantum yield of a test chemical in aqueous solution outdoors in sunlight.

a. p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR)

The p-nitroacetophenone-pyridine actinometer (PNAP/PYR) has been thoroughly described by Mill et al. (1982), Dulin and Mill (1982), and in Test Guideline CG-6000. Laboratory experiments indicated that the reaction quantum yield for the photolytic transformation of PNAP depended linearly on the molar concentration of pyridine at a fixed molar concentration of PNAP. Regression analysis of the data on the reaction quantum yield as a function of the molar concentration of pyridine [PYR] up to a concentration of 0.2 M [Winterle (1984)] (at a fixed concentration of PNAP of approximately 1 x 10^{-5} M) gave the relationship

$$\phi_a = 0.0169[PYR] \quad , \qquad (30)$$

with a correlation coefficient of 0.9986. Additional laboratory experiments indicated that ϕ_a was independent of the wavelength.

The ultraviolet-visible absorption of PNAP was obtained in an aqueous solution containing 1% acetonitrile [Mill et al. (1982] and the molar absorptivities at 313 and 366 nm (ϵ_{313a} and ϵ_{366a}) were 2,056 and 160 M^{-1} cm^{-1}, respectively. With a 450 watt medium pressure mercury lamp in an Ace-type photochemical "merry-go-round" reactor, this actinometer can be adjusted with pyridine to have half-lives that range from greater than 12 hours to several weeks (Section II.J.11.a).

b. p-Nitroanisole-Pyridine Actinometer (PNA/PYR)

The p-nitroanisole-pyridine actinometer (PNA/PYR) has been thoroughly described by Mill et al. (1982) and Dulin and Mill (1982). Laboratory photolysis experiments indicated that the reaction quantum yield for the transformation of PNA depended linearly on the molar concentration of pyridine at a fixed concentration of PNA. Regression analysis of the data on the reaction quantum yield as a function of the molar concentration of pyridine [PYR] up to a concentration of 0.02 M (at a fixed concentration of PNA of approximately 1 x 10^{-5} M) gave the relationship

$$\phi_a = 0.437[PYR] + 0.000282 \quad , \qquad (31)$$

with a correlation coefficient of 0.9998. Additional laboratory work indicated that ϕ_a was independent of the wavelength.

The ultraviolet-visible absorption spectrum of PNA was obtained in an aqueous solution containing 1% acetonitrile [Mill et al. (1982)] and the molar absorptivities at 313 and 366 nm (ε_{313a} and ε_{366a}) were 10,300 and 1,990 M^{-1} cm^{-1}, respectively. With a 450 watt medium pressure mercury lamp in an Ace-type photochemical "merry-go-round" reactor, this actinometer can be adjusted with pyridine to have half-lives that range from approximately 15 minutes to more than 12 hours (Section II.J.11.b).

2. High Optical Density Actinometers

There are three excellent high optical density actinometers (absorbance > 2) which can be used to determine the reaction quantum yield of a test chemical. These are the ferrioxalate actinometer, the Reinecke's salt actinometer, and the o-nitrobenzaldehyde actinometer. The ferrioxalate actinometer is an ideal actinometer for environmental actinometry since it is in aqueous solution and is most suitable for use in the spectral range 290-500 nm. This actinometer is highly recommended and has been adopted for use in this Test Guideline. The one disadvantage of this actinometer is that actinometry experiments must be carried out in a dark room with photographic "safe lights" since it is sensitive to visible light. The o-nitrobenzaldehyde actinometer is another excellent high optical density actinometer, but it must be used in acetonitrile. It is easy to use but is only sensitive in the range 280-400 nm. This actinometer is described in this Support Document and can be used as an optional actinometer in place of the ferrioxalate actinometer, provided the test chemical absorbs in the spectral region 290-400 nm. This actinometer follows zero-order kinetics and is applicable up to 25% transformation [Mill (1984)]. Reinecke's salt is another excellent aqueous solution actinometer which is recommended for use in the spectral region 500-750 nm. Because this actinometer is sensitive to visible light, actinometry experiments must be carried out in a dark room with photographic "safe lights". All these actinometers are high efficiency actinometers which have well defined quantum yields at fixed wavelengths.

a. Potassium Ferrioxalate Actinometer

The potassium ferrioxalate actinometer developed by Parker (1953) and Hatchard and Parker (1956) is a widely used solution phase actinometer by many photochemists. It is used at high optical density (absorbance > 2) and is applicable over a wide range of wavelengths (254-577 nm). However, it is most useful in the range 254-500 nm. Since it is an excellent actinometer and has a large wavelength range of sensitivity in the environmentally relevant region 290-500 nm, it has been adopted for use in this Test Guideline.

Quantum yields for the ferrioxalate actinometer are given in Table 1 as a function of wavelength [de Mayo and Shizuka (1976)]. At 0.15 M ferrioxalate, the quantum yield at 313 nm is 1.20 and the average quantum yield at 366 nm is 1.18. The net photochemical reaction is

$$Fe^{3+} + (C_2O_4)^{2-}/2 \xrightarrow{h\nu} Fe^{2+} + CO_2 \quad . \qquad (32)$$

From the stoichiometry of equation 32

$$-\,d[Fe^{3+}]/dt = d[Fe^{2+}]/dt \quad . \qquad (33)$$

At high optical density, equations 27, 28, and 29 are applicable and the reaction kinetics are zero-order. The kinetics of this actinometer are followed by measuring the molar concentration of Fe^{2+} formed as a function of time. The molar concentration of Fe^{2+} is measured spectrophotometrically via the formation of the red phenanthroline complex and the determination of the absorbance of the complex at 510 nm. Ferric ion forms only a weak complex with phenanthroline which is transparent at 510 nm. Murov (1973) gives a detailed procedure for using the ferrioxalate actinometer and this procedure is highly recommended. Zepp (1984) has found that it is most useful to use the ferrioxalate actinometer at a concentration of 0.15 M. At 0.15 M, the ferrioxalate actinometer can accept a much greater dose of light than at 0.006 M. The irradiated solution is diluted 100-fold prior to analysis of Fe^{2+}, which must not be allowed to exceed 0.005 M. Thus, it is recommended that the Murov procedure by modified as described by Zepp.

Table 1. Quantum Yields of Fe^{2+} (ϕ_λ) at 22°

Wavelength nm	$[K_3Fe(C_2O_4)_3]$ M	ϕ_λ
577/9	0.15	0.013
546	0.15	0.15
509	0.15	0.86
480	0.15	0.93
468	0.15	0.92
436	0.15	1.01
416	0.006	1.12[b]
	0.006	1.11
405	0.006	1.14
392	0.006	1.13[b]
365/6	0.006	1.21, 1.26[a]
	0.15	1.15, 1.20[a]
358	0.006	1.25[b]
334	0.006	1.23
313	0.006	1.24
	0.15	1.20[c]
297/302	0.006	1.24
254	0.006	1.25

---

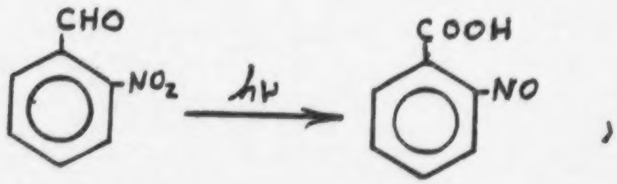
a. Lee J and Selinger HH. 1964. J Chem Phys 40:519.
b. Wegner EE and Adamson AW. 1966. J Am Chem Soc 88:394.
c. Zepp (1984).

With a 450 watt medium pressure mercury lamp filtered at 313 or 366 nm in a photochemical "merry-go-round" reactor, ferrioxalate decomposes rapidly and samples should be taken for analysis in the range of 1 to 15 minutes.

b. o-Nitrobenzaldehyde Actinometer

The o-nitrobenzaldehyde actinometer was originally developed by Pitts et al. (1964) for use in a KBr matrix. This actinometer was then used in polymethacrylate film and procedures for use in this matrix are fully described by de Mayo and Shizuka (1976).

Smith et al. (1977, 1978) described the use of the p-nitrobenzaldehyde actinometer in solution with acetonitrile as the solvent. Dulin and Mill (1982) reported the use of this actinometer in acetonitrile as a high optical density actinometer. The photochemical reaction is



(34)

and the reaction quantum yield was reported to be 0.505 and is independent of the wavelength. It is sensitive in the spectral region 280-400 nm. The photochemical reaction was studied by measuring the concentration of o-nitrobenzaldehyde (o-NB) remaining at time t using reverse-phase high pressure liquid chromatography using 50% acetonitrile in water as the eluent. In the calibration experiments by Dulin and Mill (1982), a solution of approximately 1×10^{-2} M o-NB in acetonitrile was photolyzed in quartz or borosilicate tubes for periods up to 16 minutes at 313 and 366 nm in an Ace-type photochemical "merry-go-round" reactor with a 450 watt medium pressure mercury lamp. They reported that the light intensity I_λ at 313 nm varied from 2.5×10^{-6} to 3.5×10^{-6} einsteins sec.^{-1} liter^{-1} and at 366 nm I_λ varied from 6.7×10^{-6} to 8.2×10^{-6} einsteins sec.^{-1} liter^{-1}. Mill (1984) reported that the zero-order kinetics were obeyed up to 25% transformation and the molar absorptivities at 313 and 366 nm (ε_{313a} and ε_{366a}) were 1500 and 181 M^{-1} cm^{-1}, respectively. At 313 nm all the light is absorbed by o-NB in a 1 cm cell. At 366 nm, essentially all the light is absorbed by o-NB in a 1 cm cell (98.5% of the light is absorbed at 366 nm). If this actinometer is used at $\lambda >$ 366 nm to 400 nm, the concentration of o-NB must be adjusted in the reaction cell so that the optical density is > 2 in the reaction cell throughout irradiation.

The o-nitrobenzaldehyde actinometer in acetonitrile solution is an excellent actinometer for use in laboratory photolysis experiments. It is easy to use but has the limitation that it is only sensitive in the spectral region 280-400 nm. It is highly recommended for use with test chemicals that absorb in the region 290-400 nm. It is described in detail in this section but not in the Test Guideline. If this actinometer is used in the Test Guideline, then the Test Data Report of Test Guideline CG-6010 should state it. It should be noted that since the photochemical reaction is followed by measuring the concentration of o-nitrobenzaldehyde as a function of time, equations 23, 24, and 25 are applicable.

c. Reinecke's Salt Actinometer

The ferrioxalate actinometer is a very useful actinometer but has the limitation that it is most useful in the environmentally relevant spectral range 290-500 nm. The Reinecke's salt actinometer [$KCr(NH_3)_2(NCS)_4$], developed by Wegner and Adamson (1966), can be used in the spectral range 500-750 nm. Thus, it is recommended for use to determine the reaction quantum yield of test chemicals that absorb in the range 500-750 nm.

Upon photolysis of this actinometer, the Reinecke's salt gives the hydrate $Cr(NH_3)_2(NCS)_3(H_2O)$ and NCS^-. The formation of thiocyanate ion can be determined with ferric ion spectrophotometrically. The actinometer is used in aqueous solution with an optical density ≥ 2.2. De Mayo and Shizuka (1976) give a detailed procedure for using this actinometer and this procedure is highly recommended.

D. Determination of the Cell Pathlength

From the theoretical discussion given in Section II.B, it is evident that the pathlength of the light is an important variable, especially when low optical density actinometers are used (equation 18). For a 1-cm square cell the pathlength is easily defined. However, for a cylindrical cell where the light falls on rounded surfaces, the pathlength is not easily defined because of refraction and reflection in the cell. Zepp (1978) developed an experimental method for determining the effective pathlength of any cell, and in particular for cylindrical cells which are used in environmental studies.

If the molar concentration (C) of the light absorbing substance is high, then $\varepsilon_\lambda C >> \alpha_\lambda$ and to a good approximation $(\alpha_\lambda + \varepsilon_\lambda C) = \varepsilon_\lambda C$. Using this result in equations 9 and 10 yields

$$\begin{aligned} F_{s\lambda} &= 1 - 10^{-\varepsilon_\lambda C\ell} \\ F_{c\lambda} &= 1 \quad . \end{aligned} \tag{35}$$

Under these conditions, equation 11 becomes

$$(\text{Rate})_C = -(dC/dt) = \phi I_\lambda (1 - 10^{-\varepsilon_\lambda C\ell}) \quad . \tag{36}$$

For the special case when essentially all the light is absorbed by the chemical, $\varepsilon_\lambda C\ell > 2$ and $10^{-\varepsilon_\lambda C\ell} \longrightarrow 0$. Equation 36 becomes

$$(\text{Rate})_{\text{max.}} = \phi I_\lambda \quad . \tag{37}$$

Dividing equation 37 into 36 yields

$$(\text{Rate})_C/(\text{Rate})_{\text{max.}} = 1 - 10^{-\varepsilon_\lambda C\ell} = X \quad . \tag{38}$$

Rearranging equation 38 yields

$$1 - X = 10^{-\varepsilon_\lambda C\ell}$$

and

$$-\log_{10}(1 - X) = \varepsilon_\lambda C\ell \quad . \tag{39}$$

A plot of $-\log_{10}(1 - X)$ vs. $\varepsilon_\lambda C$ yields a straight line with a slope equal to the pathlength of the cell ℓ. Zepp (1978) measured the effective cell pathlength of small cylindrical cells used in his environmental photochemical studies by employing the benzophenone-sensitized isomerization of cis-1,3-pentadiene. This procedure has been used to measure the effective pathlength of Corning Glass culture tubes 13 x 100 nm and it was found that ℓ was 11.2 mm. [Mill et al. (1982a)]. In a similar manner, reaction tubes made from borosilicate stock of 12 mm o.d. had an effective pathlength of 10.0 mm. [Mill et al. (1982a)].

The following procedure can be used to determine the cell pathlength of cylindrical cells using the o-nitrobenzaldehyde actinometer (o-NB) which is described in Section II.C.2.b.

1. Prepare o-NB solutions with an optical density > 2 with known concentration C_m.
2. Prepare, by sequential dilutions of the solution above, o-NB solutions with optical densities 1 to 0.1 (e.g., OD's approximately 1, 0.8, 0.6, 0.4, 0.2, and 0.1) with known concentrations C_1, C_2, C_3, C_4, C_5, and C_6.
3. Photolyze the seven solutions (C_m, C_1, C_2, C_3, C_4, C_5, C_6) for identical times in a PMGRR and analyze for o-NB by the procedure outlined in Section II.C.2.b. Reaction should correspond to less than 25% transformation of o-NB.
4. Determine the ratios $\Delta C_1/\Delta C_m$, $\Delta C_2/\Delta C_m$, etc.... These ratios are designated X_1, X_2, etc... .
5. Plot of the quantity $[-\log_{10}(1-X_1)]$ versus $\varepsilon_\lambda C_1$, $[-\log_{10}(1-X_2)]$ versus $\varepsilon_\lambda C_2$, etc. for each set of data points; ε_λ is the absorption coefficient for o-NB at the photolysis wavelength λ .
6. Calculate the slope of the best straight line which is equal to the effective cell pathlength ℓ [equation 39]. A more accurate and precise value of ℓ may be obtained by a fit of the data to equation 39 using linear regression analysis.

These photolysis experiments can be carried out at wavelengths 313 or 366 nm. The molar absorptivities of o-NB at these wavelengths are: $\varepsilon_{313a} = 1500\ M^{-1}cm^{-1}$ and $\varepsilon_{366a} = 181\ M^{-1}cm^{-1}$ [Mill (1984)].

When reaction quantum yield experiments are carried out in cylindrical cells (i.e., tubes) using different solvents for actinometer and test chemical, the light flux measured, and thus the reaction quantum yield of the test chemical in an Ace-type photochemical "merry-go-round" reactor, will be solvent dependent because of the different refractive indices of the solvents used. Vesley (1971) measured the reaction quantum yield for the photoisomerization of cis-1,3-pentadiene in benzene at 365 nm in an Ace-type photochemical "merry-go-round" reactor using the standard actinometers ferrioxalate in water and benzophenone-benzhydrol in benzene. The reaction quantum yield of the photoisomerization reaction was found to be 0.64 when the ferrioxalate actinometer (in water) was used while the reaction quantum yield was found to be 0.55 when the benzophenone-benzhydrol actinometer (in benzene) was used. Vesley attributed the differences in the values of the reaction quantum yield to the differences in refractive indices of water and benzene relative to the refractive index of glass. Benzene has a refractive index $n^{20°}=1.501$ which is similar to the refractive index of pyrex $n^{20°}=1.52$ and thus all the incident light passed into the solution whereas with water, with a refractive index $n^{20°}=1.332$, some of the light is refracted and reflected at the inner cell walls. As a result, the samples in which the benzene was used as the solvent "see" more photons than the aqueous actinometer solutions. Thus, the ferrioxalate actinometer underestimated the light that actually entered the solution in the cell when benzene was the solvent for the photoisomerization reaction and thus overestimated the true reaction quantum yield for the cis-1,3-pentadiene by 15%.

Since in environmental photochemistry water is the medium in which photolysis takes place,it is important that actinometry experiments be performed in aqueous solution whenever practical. In those cases where aqueous actinometers are not practical, the actinometer solvent actonitrile ($n^{20°}$=1.344) may be used since its refractive index is close to that of pure water.

E. Development of the Test Method

In general, the reaction quantum yield is determined under the experimental conditions in which all the incident light is absorbed by the chemical (absorbance > 2). The concentrations of chemicals used in these experiments range from 10^{-3} M to greater than 0.1 M. However, most organic pollutants are hydrophobic so that the water solubility is generally below 10^{-3} M. Hence, for these chemicals in aqueous solution, only a small fraction of the incident light is absorbed (absorbance < 0.02). Zepp (1978) described two procedures for determining the reaction quantum yield of organic pollutants in very dilute aqueous solution with an absorbance < 0.01. One procedure measures the light intensity incident on the sample using a high optical density actinometer (e.g., the ferrioxalate actinometer). This procedure is used extensively by photochemists and therefore it has been incorporated in this Test Guideline.

In addition, Zepp (1978) described a second procedure for determining the reaction quantum yield by comparing the photolysis rate of the pollutant and actinometer under conditions in which the light is only weakly absorbed by both compounds. Such experiments are conveniently made in a photochemical "merry-go-round" reactor. This elegant method has not been used because the standard chemical actinometers cannot be analyzed at the low concentrations required for this technique. However, two excellent low optical density variable reaction quantum yield actinometers have been recently developed for the Office of Toxic Substances/U.S. Environmental Protection Agency by SRI International [Mill et al. (1981) and Dulin and Mill (1982)] and these actinometers are discussed in Section II.C.). One of these actinometers (PNAP/PYR) has already been incorporated in Test Guideline CG-6000. Thus, this second procedure has also been incorporated in this Test Guideline (CG-6010).

There is another procedure which can be used to determine the reaction quantum yield of a test chemical at high optical density by measuring the light incident on the test chemical with a high optical density actinometer (e.g., the ferrioxalate actinometer). This excellent procedure, however, has only limited applicability to those test chemicals which are very soluble in water and have high optical density (absorbance > 2). Thus, this procedure has not been incorporated in this Test Guideline. However, it is discussed in this Support Document and may be used for certain organic pollutants. If this procedure is used for these pollutants, then it must be described in detail.

1. Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method

a. Theoretical Discussion

Since aqueous solutions of the test chemical and actinometer will be photolyzed at low optical density (absorbance < 0.02), equation 18 is applicable. Using this equation, the equations for test chemical (c) and actinometer (a) can be written as follows:

$$k_{pc} = 2.303\phi_c I_\lambda \varepsilon_{\lambda c} \ell \qquad (40)$$

$$k_{pa} = 2.303\phi_a I_\lambda \varepsilon_{\lambda a} \ell \quad . \qquad (41)$$

If the aqueous solutions of test chemical and actinometer are photolyzed simultaneously in identical cells with the same pathlength ℓ and at a fixed wavelength λ (or in a narrow wavelength band) in a photochemical "merry-go-round" reactor (PMGRR), then equation 41 can be divided into 40. Carrying out this operation and rearranging the result yields

$$\phi_c = (k_{pc}/k_{pa})(\varepsilon_{\lambda a}/\varepsilon_{\lambda c})\phi_a \quad . \qquad (42)$$

Using equation 19, the equations for test chemical (c) and actinometer (a) can be written as follows:

$$\ell n(C_o/C_t)_c = k_{pc}t \qquad (43)$$

$$\ell n(C_o/C_t)_a = k_{pa}t \quad ; \qquad (44)$$

the half-life of the test chemical (c) and actinometer (a) can be written as $(t_{1/2})_c$ and $(t_{1/2})_a$ and can be obtained from equation 20. Again, since both the actinometer and the test chemical solutions are photolyzed simultaneously in identical cells in a PMGRR, then equation 44 can be divided into equation 43. Carrying out this operation and rearranging the result yields

$$\ell n(C_o/C_t)_c = (k_{pc}/k_{pa})\ \ell n(C_o/C_t)_a \quad . \qquad (45)$$

During photolysis at wavelength λ , the concentrations of test chemical and actinometer are measured periodically as a function of the time t. These data are then used to determine the ratio of the rate constants (k_{pc}/k_{pa}) using linear regression analysis on equation 45. The slope of the best straight line is the ratio of the rate constants.

Using spectroscopic procedures, the molar absorptivities of test chemical and actinometer ($\varepsilon_{\lambda c}$ and $\varepsilon_{\lambda a}$) can be determined at the absorbing wavelength λ. Combining these data along with the ratio of the rate constants (k_{pc}/k_{pa}) and the reaction quantum yield of the actinometer (ϕ_a), the reaction quantum yield of the test chemical (ϕ_c) can be determined using equation 42.

b. Procedure One: Determination of the Reaction Quantum Yield by the Simultaneous Photolysis of Test Chemical and Actinometer at Low Optical Density

Procedure One involves the simultaneous photolysis of aqueous solutions of test chemical and actinometer at low optical density (absorbance < 0.02) in an Ace-type PMGRR* to determine the reaction quantum yield of the test chemical. The excitation source is a 450 watt medium pressure mercury lamp with appropriate filters to isolate the 313 or 366 nm bands. The procedure has been divided into three phases. In Phase 1, the molar absorptivities of test chemical (c) are determined at 313 and 366 nm (ε_{313c} and ε_{366c}) using uv-visible absorption spectroscopy. Based on these results, photolysis experiments are carried out at 313 or 366 nm corresponding to the higher value of ε_{313c} or ε_{366c}. The Phase 2 procedure is composed of trial experiments in a PMGRR at 313 or 366 nm to determine the approximate rate constant and half-life of the test chemical and to choose the appropriate actinometer which has a rate constant approximately the same as the rate constant of the test chemical. In the Phase 3 procedure, aqueous low optical density solutions of test chemical and actinometer (at a fixed molar concentration of pyridine [PYR]) are photolyzed simultaneously in a PMGRR at 313 or 366 nm. The data obtained from the Phase 1, 2, and 3 experiments are then used to determine the reaction quantum yield of the test chemical.

---

*The Ace-type PMGRR is designed so that the entire reaction cell is irradiated. This type of PMGRR is commercially available. There is a Moses-types PMGRR [Moses et al. (1969)] which differs slightly from the Ace-type PMGRR in that it contains windows (i.e., slits) so that only a narrow portion of the solution in the reaction cell is irradiated. For details see Section II.J.2.a and II.J.11.c.

i. Phase 1 Experiments

In the Phase 1 experiments, the molar absorptivities of the test chemical (c) are determined at 313 and 366 nm (ε_{313c} and ε_{366c}) using procedures outlined in Test Guidelines CG-1050 and CG-6000. The method outlined in these two guidelines is the standard procedure used by all laboratories for determining the molar absorptivity of a chemical in solution. Photolysis experiments will then be carried out in the Phases 2 and 3 experiments at 313 or 366 nm corresponding to the higher value of ε_{313c} or ε_{366c}. By photolyzing the test chemical at the wavelength corresponding to the higher value of molar absorptivity, the rate of photolysis is maximized.

ii. Phase 2 Experiments

First, a dilute aqueous solution of test chemical (c) at a low optical density (absorbance < 0.02) and at an initial concentration $(C_o)_c$ is photolyzed in an Ace-type PMGRR at wavelength 313 or 366 nm (irradiation wavelength chosen based on the Phase 1 experiments) using a 450 watt medium pressure mercury lamp and appropriate filters to isolate the monochromatic wavelength λ. After approximately 50% transformation, the concentration of test chemical $(C_t)_c$ is measured at time t. These data are used in equation 43 to determine an approximate rate constant k_{pc}. This result is used in equation 20 to obtain the half-life of the test chemical $(t_{1/2})_c$. If $(t_{1/2})_c$ is less than 12 hours, use the actinometer PNA/PYR (Sections II.C.1.b and II.J.11.b). If $(t_{1/2})_c$ is greater than 12 hours, then use the actinometer PNAP/PYR (Sections II.C.1.a and II.J.11.a).*

Utilizing equation 44, trial experiments are carried out with the appropriate actinometer, at an absorbance < 0.02, to determine the molar concentration of pyridine [PYR] needed to make the rate constant of the actinometer approximately equal to the rate constant of the test chemical (within ± 50%). For more details, see Section II.J.11.

---

*If a Moses-type PMGRR is used, then new criteria have to be defined to determine which of these two actinometers have to be used. For details see Section II.J.11.c.

According to Sections II.C.1.a, b, the reaction quantum yield of these actinometers is a function of the molar concentration of pyridine; and these relationships are given by equations 30 and 31:

PNAP/PYR actinometer $\phi_a = 0.0169[PYR]$ (30)

PNA/PYR actinometer $\phi_a = 0.437[PYR] + 0.000282,$ (31)

where [PYR] is the molar concentration of pyridine for a fixed concentration of PNAP or PNA of approximately 1×10^{-5} M.

iii. Phase 3 Experiments

An aqueous solution of test chemical at low optical density and at a concentration $(C_o)_c$ is prepared and placed in small cylindrical tubes. Based on the results of the Phase 2 experiments, the appropriate actinometer is chosen and the concentration of pyridine is adjusted so that the rate constant of the actinometer is approximately equal to the rate constant of the test chemical (i.e., within ± 50%). An aqueous actinometer solution is prepared at concentration (C_o) with a molar concentration of pyridine [PYR]. The actinometer solution is placed in identical tubes used for the test chemical solution. The volumes of actinometer and test chemical solution should be the same. Aqueous solutions of test chemical and actinometer are simultaneously photolyzed in the PMGRR at the chosen wavelength 313 or 366 nm using the 450 watt medium pressure lamp and appropriate filters. The concentrations of test chemical $(C_t)_c$ and actinometer $(C_t)_a$ are measured as a function of the time t. Using these data in equation 45 and linear regression analysis, the ratio of the rate constants (k_{pc}/k_{pa}) is obtained. The reaction quantum yield of the actinometer (ϕ_a) can be determined at the molar concentration of pyridine employed in this experiment (i.e., substituting [PYR] in equation 30 or 31). Using these data along with the molar absorptivities of test chemical $(\varepsilon_{313c}$ or $\varepsilon_{366c})$ and the actinometer $(\varepsilon_{313a}$ or $\varepsilon_{366a})$, given in Sections II.C.1.a, b, in equation 42, the reaction quantum yield of the test chemical can be determined.

2. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method

a. Theoretical Discussion

Since the test chemical (c) is at low optical density, equations 18 and 19 are applicable:

$$k_{pc} = 2.303\phi_c I_\lambda \varepsilon_{\lambda c} \ell \qquad (18)$$

$$\ell n(C_o/C_t)_c = k_{pc} t \quad . \qquad (19)$$

Furthermore, since the actinometer is at high optical density and the ferrioxalate actinometer (Section II.C.2.a) is widely used by photochemists, equations 28 and 29 are applicable:

$$(C_t)_a = k_{pa}t \tag{28}$$

$$k_{pa} = \phi_a I_\lambda \quad , \tag{29}$$

where $(C_t)_a$ now represents the product formed which is $[Fe^{2+}]$. Solving for ϕ_c in equation 18 and I_λ in equation 29 yields the following two equations

$$\phi_c = k_{pc}/[2.303\ I_\lambda \varepsilon_{\lambda c} \ell] \tag{46}$$

$$I_\lambda = k_{pa}/\phi_a \quad ; \tag{47}$$

and substituting equation 47 in equation 46 yields

$$\phi_c = \phi_a(k_{pc}/k_{pa})(2.303\varepsilon_{\lambda c}\ell)^{-1} \quad . \tag{48}$$

b. Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method

Procedure Two involves the photolysis of an aqueous solution of test chemical at low optical density and an aqueous solution of an actinometer at high optical density in a photochemical optical bench (POB) or in a PMGRR to determine the reaction quantum yield of a test chemical. The excitation source is a 450 watt medium pressure mercury lamp with appropriate filters to isolate the 313 or 366 nm bands. The procedure has been divided into two phases. In the Phase 1 experiments, the molar absorptivities of test chemical (ε_{313c} and ε_{366c}) are determined at 313 and 366 nm using uv-visible absorption spectroscopy. Based on these results, photolysis experiments are carried out at 313 or 366 nm corresponding to the higher value of ε_{313c} or ε_{366c}. In the Phase 2 experiments, aqueous low optical density solutions of test chemical and aqueous high optical density solutions of the ferrioxalate actinometer are photolyzed sequentially in a POB or in a PMGRR at 313 or 366 nm. These data, along with the Phase 1 data, are used to determine the quantum yield of the test chemical.

i. Phase 1 Experiments

The phase 1 experiments are identical to those described in Section II.E.1.b.i.

ii. Phase 2 Experiments

In the first series of experiments, a dilute aqueous solution of the ferrioxalate actinometer (a) at high optical density (i.e., at absorbance > 2) is placed in a cell (or cells) and photolyzed in a POB (or a PMGRR) at wavelength 313 or 366 nm (based on the results of the Phase 1 experiments) using a 450 watt medium pressure mercury lamp and appropriate filters. The molar concentration of ferrous ion formed $(C_t)_a$ is measured as a function of the time t. These data are fitted to equation 28 using linear regression analysis to obtain k_{pa}. No measurements should be used when the absorbance of the actinometer falls below 2. In a third set of experiments at the end of this procedure, k_{pa} is determined again in the same manner as described above. The results of these two experiments are averaged to obtain $(k_{pa})_{ave.}$.

In a second set of experiments, a dilute aqueous solution of test chemical (c) at low optical density (absorbance < 0.02) and at an initial concentration $(C_o)_c$ is then photolyzed in the same POB or PMGRR. The reaction cell or cells must be identical to those used in the actinometry experiments and contain the same volume of solution. The concentration of test chemical $(C_t)_c$ is measured as a function of time and these data are fitted to equation 19 using linear regression analysis to obtain k_{pc}. Using k_{pc} and $(k_{pa})_{ave.}$, the molar absorptivity of the test chemical (ε_{313c} or ε_{366c}), and the pathlength ℓ of the reaction cell or cells (which must be known or determined by the procedure described in Section II.D) in equation 48, the reaction quantum yield of the test chemical (ϕ_c) can be determined.

3. Procedure Three: Determination of the Reaction Quantum Yield by the High Optical Test Chemical and Actinometer Method

Since the test chemical (c) and actinometer (a) solutions will be photolyzed at high optical density (absorbance > 2), equation 25 is applicable. Using this equation, the equations for test chemical (c) and actinometer (a) can be written as follows:

$$k_{pc} = \phi_c I_\lambda \tag{49}$$

$$k_{pa} = \phi_a I_\lambda \quad . \tag{50}$$

If aqueous solutions of the test chemical and actinometer are photolyzed simultaneously in identical cells at a fixed wavelength λ in a PMGRR, then equation 50 can be divided into equation 49. Carrying out this operation and rearranging the result yields

$$\phi_c = \phi_a (k_{pc}/k_{pa}) \quad . \tag{51}$$

Assuming that the concentration of test chemical and actinometer are measured as a function of time during photolysis, then equation 24 is applicable. Thus, the equations for test chemical (c) and actinometer (a) can be written as follows:

$$(C_o - C_t)_c = k_{pc}t \qquad (52)$$

$$(C_o - C_t)_a = k_{pa}t \quad . \qquad (53)$$

Since both the test chemical and actinometer solutions are photolyzed at wavelength λ in a PMGRR in identical cells, then equation 55 can be divided into equation 54. Carrying out this operation and rearranging the result yields

$$(C_o - C_t)_c = (k_{pc}/k_{pa})(C_o - C_t)_a \quad . \qquad (54)$$

During the photolysis, the molar concentrations of test chemical and actinometer are measured periodically as a function of time t. These data are then used to determine the ratio of the rate constants (k_{pc}/k_{pa}) using linear regression analysis on equation 54. The slope of the best straight line is the ratio of the rate constants.

If it is now assumed that the high optical density actinometer is the ferrioxalate actinometer, then equation 28 is applicable. The equations for test chemical (c) and actinometer (a) can be written as follows:

$$(C_o - C_t)_c = k_{pc}t \qquad (52)$$

$$(C_t)_a = k_{pa}t \quad ; \qquad (55)$$

and dividing equation 55 into equation 52 and rearranging the result yields

$$(C_o - C_t)_c = (k_{pc}/k_{pa})(C_t)_a \quad . \qquad (56)$$

If now the molar concentration of ferrous ion $(C_t)_a$ is measured along with the molar concentration of test chemical $(C_t)_c$ as a function of the time t, then these data can be substituted into equation 56 and the ratio of the rate constants (k_{pc}/k_{pa}) can be obtained using linear regression analysis.

Finally, using the ratio of the rate constants (k_{pc}/k_{pa}) as described above and the reaction quantum yield of the actinometer (ϕ_a) at wavelength λ in equation 51, the reaction quantum yield of the test chemical (ϕ_c) can be obtained.

Another procedure can be used to determine the reaction quantum yield of a test chemical (ϕ_c) by sequentially photolyzing actinometer and test chemical solutions. In this method, aqueous solutions of test chemical and actinometer at high optical density (absorbance > 2) are photolyzed sequentially at a wavelength λ in a POB (or PMGRR). In the first series of experiments, the actinometer solution is photolyzed in a cell (or cells) in a POB (or PMGRR) and the molar concentration of actinometer (a) is measured as a function of the time t. Using these data in equation 53 and linear regression analysis, the actinometer rate constant is obtained since the slope equals k_{pa}. If the ferrioxalate actinometer is used, then equation 55 is applicable. By measuring the concentration of ferrous ion formed $(C_t)_a$ as a function of the time t, k_{pa} can then be determined using these data in equation 55 and linear regression analysis. In the third series of experiments, the above procedure is repeated and another actinometer rate constant is obtained. The results of these two experiments are averaged to yield $(k_{pa})_{ave.}$. Since the reaction quantum yield of the actinometer (ϕ_a) is known at wavelength λ, and the actinometer rate constant $(k_{pa})_{ave.}$ has been obtained by the procedure described above, then I_λ can be determined using equation 50.

In the second series of experiments, an aqueous solution of test chemical at high optical density is photolyzed at wavelength λ in a cell (or cells) in a POB (or PMGRR). The cell (or cells) must be identical to those used in the actinometer experiments and contain the same volume of solution. The molar concentration of test chemical is measured as a function of the time t and these data are used in equation 52 to obtain k_{pc} using linear regression analysis. Using the values of k_{pc} and I_λ, as described above, in equation 49, the reaction quantum yield of test chemical (ϕ_c) can be determined.

F. Quantum Yield as a Function of Wavelength

In Section II.A, it has been shown that the rate constant k_{pE} in the environment is a function of the reaction quantum yield and it has been assumed that the reaction quantum yield is independent of the wavelength. This assumption is valid for most complex molecules in solution because the photoreaction from second or higher electronic states usually cannot compete with the rapid radiationless decay of excited molecules to their first excited state [Zepp (1978)]. Thus, this Test Guideline only recommends measuring the reaction quantum yield of a test chemical at one wavelength. However, there are exceptions to this rule (e.g., organometallics) and this must be considered when carrying out these experiments. Determination of the reaction quantum yield at more than one wavelength may be discussed in a Test Rule for specific chemicals or classes of chemicals.

G. Photolysis and Molar Absorptivity in Water

Since water is the environmentally relevant medium in which photolysis takes place, these reaction quantum yield experiments should be carried out in pure water. Up to 1% acetonitrile, however, is acceptable. Furthermore, whenever possible, the molar absorptivity of the test chemical should be determined in pure water. However, determination of the molar absorptivity is difficult for hydrophobic chemicals and is essentially impossible for very hydrophobic chemicals (e.g., DDT, PCBs, and polyaromatic hydrocarbons). As an alternative, Zepp(1978) recommended the determination of the molar absorptivity in mixtures of water and acetonitrile or methanol. Since these solvents are effective in dissolving many very hydrophobic organic chemicals, have refractive indices very close to that of water and thus cause minimal solvent effects (i.e., minimum shifts and changes in the absorbance of strong absorption bands of non-polar compounds), and do not absorb radiation at wavelengths greater than 290 nm, they have been recommended for use in this Test Guideline for determining the molar absorptivity of test chemicals. However, a minimum amount of the solvent should be used to measure the molar absorptivity of a test chemical.

H. Solar Irradiance Data

In order to calculate the sunlight direct photolysis ratio constant k_{pE} and the corresponding half-life $t_{1/2E}$ for a test chemical as a function of latitude and season of the year anywhere in the United States, it is necessary to use the solar irradiance parameter L_λ . L_λ values are proportional to the average light flux that is available to cause photolysis in the wavelength interval λ over a 24-hour day at a specific latitude and season date. The L_λ values are defined by the angle of declination of the sun at -20° for winter, -10° for fall, +10° for spring and +20° for summer. The actual dates for 1982 that correspond to these angles of declination are January 21, April 16, July 24, and October 20, for winter, spring, summer and fall, respectively [AA (1982)]. The L_λ values are listed in Tables 3 to 6 of Test Guideline CG-6000 as a function of latitude and season of the year and are applicable to clear sky conditions, shallow depths in water bodies, and for chemicals whose absorbance is less than 0.02 in pure water [Mill et al. (1982a)]. The theoretical basis for the L_λ data is discussed in Section II.D.9 of Technical Support Document CS-6000.

I. Applicability and Specificity of the Test Method

Because of the theoretical principles outlined in Section II.E, Procedure One is only applicable to test chemicals and actinometers which have low optical density (i.e., absorbance < 0.02) while Procedure Two is only applicable to a test chemical with low optical density and with actinometers at high optical density (i.e., absorbance > 2).

For environmental photochemistry, the general procedures outlined in this Test Guideline are applicable to all chemicals which have uv-visible absorptions in the region 290-800 nm. Solar radiation reaching the earth's surface has a sharp cutoff at a wavelength of approximately 290 nm due to the absorption by ozone [Leighton (1961), Peterson (1976), Zepp and Cline (1977), Demerjian (1980)]. The long wavelength limit is set by thermochemistry since light of wavelength greater than 800 nm is not of sufficient energy to break chemical bonds of ground state molecules [Calvert and Pitts (1966), Benson (1976)]. Photolysis does not occur unless there is absorption of radiant energy. This is the direct consequence of the Grotthus-Draper law, the first law of photochemistry. If a chemical in aqueous solution only absorbs light at wavelengths below 290 nm it will not undergo direct photolysis in sunlight. A few examples of chemicals that only absorb light below 290 nm and therefore should not be tested by this Test Guideline are alkanes, alkenes, alkynes, saturated alcohols, and saturated acids. It is possible that some chemicals will absorb radiation mainly at wavelengths below 290 nm but may have an absorption tail that extends above 290 nm. Photolysis experiments should be carried out for these chemicals.

Procedure One, as described in this Test Guideline, is limited to the wavelength region 290-400 nm because the only well developed low optical density actinometers are sensitive in this region (Sections II.C.1.a, b). Fortunately, this disadvantage is minimal since a large number of chemicals absorb light in this spectral region.

Procedure Two, as described in this Test Guideline, is applicable to the wavelength region 290-750 nm. The ferrioxalate actinometer is the most useful in the region 290-500 nm (Section II.C.2.a) while the Reinicke's salt actinometer is useful in the region 500-750 nm (Section II.C.2.c).

This Test Guideline is only applicable to pure chemicals and not to the technical grade. Overestimates of molar absorptivities usually occur when technical grade substances are tested because the impurities frequently absorb in the same spectral region as the pure chemical.

J. Rationale for the Section of the Test Conditions

1. UV-Visible Absorption Spectrometer

All the laboratory equipment needed to determine the uv-visible absorption spectrum of a test chemical in aqueous solution is listed in the references in Test Guideline CG-1050. The equipment required to carry out this test is standard equipment which is commonly used in most laboratories.

2. Special Photochemical Laboratory Equipment

a. Design of Apparatus

The apparatus used to measure photochemical kinetics in the laboratory is usually the "merry-go-round" reactor (PMGRR) or the optical bench (POB). There are two types of PMGRR equipment; the Ace-type and the Moses-type. The Ace-type PMGRR is designed so that the entire reaction cell is irradiated while the Moses-type PMGRR [Moses et al. (1969)] is designed with windows (which act as slits with a fixed aperture) so that only a narrow portion of the reaction cell is irradiated. Only the Ace-type is commercially available from Ace Glass Company, Vineland, New Jersey; the Moses-type PMGRR would have to be specially built in a machine shop. Since the Ace-type PMGRR is the only one commercially available, this Test Guideline has been specifically designed to use this PMGRR. However, if the Moses-type PMGRR is available, this Test Guideline has to be modified slightly as described in Section II.J.11.c.

POB equipment is commercially available from a number of sources. One type of POB which can be used is a Schoeffel Reaction Chemistry System [Schoeffel Industries, Inc.] which contains a 1000 watt xenon lamp, a high intensity monochromator, and a sample compartment.

Both PMGRR and POB equipment provide for constant and uniform irradiation of a solution at a fixed distance from the light source and allow for the use of a light-filter system between the light sources and the reaction solution. These two pieces of equipment are standard equipment used by photochemists to study photochemical reaction kinetics in the laboratory and are described in numerous sources in the literature [e.g., Calvert and Pitts (1966), de Mayo and Shizuka (1976), Murov (1973), Zepp (1982), Moses et al. (1969), and Mill et al. (1982a)].

b. Light Sources

There are a number of light sources which are available for use in photochemical studies (e.g., low, medium, and high pressure mercury lamps; xenon lamps; and lasers). The selection of the specific light sources should be determined by the following criteria [de Mayo and Shizuka (1976)]:

(1) The wavelength of the light absorbed by chemicals(λ) should be of sufficient intensity to induce photolysis in a reasonable amount of time. The intensity of the light source should be greater than 10^{14} quanta/sec./cm^2.

(2) The light source should be stable for prolonged periods of irradiation.

(3) If possible, the light emanating from the light source should be a point source for ease of collimation.

(4) A monochromatic light source is desirable since the excitation wavelength may be a narrow band without the loss of intensity.

The 450 watt medium pressure mercury lamp has most of these characteristics and is widely used by many photochemists. The PMGRR is designed to accomodate this lamp. Therefore, it has been recommended for use in this Test Guideline. Numerous other light sources are available and may be used in this Test Guideline. These light sources are described in detail by Calvert and Pitts (1966), de Mayo and Shizuka (1976), and Murov (1973). If a light source other than the 450 watt medium pressure mercury lamp is used, it must be completely described in the Test Data Report Section of this Test Guideline.

c. Light Filtering Systems

Monochromatic or narrow band wavelength light is essential for the accurate determination of the reaction quantum yield of a test chemical by the procedures outlined in this Test Guideline [see the theoretical discussions in Sections II.B. and E]. A serious error can arise with an incorrectly filtered light source that allows light (i.e., leak light) at other than the irradiation wavelength λ . If $\varepsilon_{\lambda C}$ is dramatically larger at the leak wavelength, a small fractional leak can produce a large error because the fraction of the light absorbed is a linear function of $\varepsilon_{\lambda}c$.

Because the reaction quantum yields of pollutants are usually low,it is important to have high intensity monochromatic light so that faster photolysis rates are obtained in the laboratory equipment. High intensity grating monochromators can be used to isolate narrow-band radiation from an intense uv source such as mercury or xenon lamps; however, this equipment is expensive. Alternatively, a narrow wavelength band can also be isolated by the use of chemical solutions and/or glass filters; and a number of these combinations have been described by Calvert and Pitts (1966), Murov (1973), de Mayo and Shizuka (1976), and Mill et al. (1982a). These filter systems are inexpensive. Glass interference filters are also very efficient in isolating narrow wavelength bands of light.

Two filter systems are described in this Test Guideline to isolate the 313 and 366 nm bands from a 450 watt medium pressure mercury lamp. These filter systems are inexpensive and are commonly used by many photochemists. Hence, they are highly recommended and have been incorporated for use in this Test Guideline. For the 313 nm filter system, it is recommended that a 0.005 M solution of potassium chromate be used to filter out the 302 nm mercury band. If the test chemical does not absorb light at wavelengths greater than 400 nm, the glass filter Corning CS-754 is not needed. The 754 filter is designed to block out visible light. If other light filtering systems are used other than those recommended in the Test Guideline, then they must be completely described in the Test Data Report Section of this Test Guideline. For these light filtering systems, it is important to check the light emanating from them with a monochromator to make sure that the desired wavelength band has been isolated.

d. Reaction Cells

In general, reaction cells of large volume are appropriate for POB equipment while small reaction cells are used for the PMGRR equipment. Small volume reaction cells (e.g., rectangular cuvettes of approximately 4 mL) can be used with the Schoeffel Reaction Chemistry System [Zepp (1984)]. Test chemical and actinometer reaction solutions must be photolyzed in identical cells [see the theoretical discussions in Sections II.B.and E].

For the PMGRR equipment, small tubes are required because of the nature of the design of this equipment. These tubes can be constructed from quartz or borosilicate glass. Reaction tubes with an inside diameter of 11 mm are recommended as they are inexpensive and are easy to seal to prevent volatilization of the test chemical. Grease should be avoided since hydrophobic chemicals might adsorb to it. Disposable culture tubes (13 x 100 mm) with Teflon-lined screw caps are recommended since they are inexpensive, easy to seal, and are readily available from commercial laboratory supply companies. Volatile compounds can be conveniently studied in culture tubes equipped with gas-tight Mininert® valves. Sampling can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate [Zepp (1984)].

For the POB equipment, the most common and functional design for reaction cells is a cylindrical shape with optically flat circular windows fused to each end of the cylinder and at right angles to its axis. Optically flat quartz windows are recommended because they transmit 100% of the light at 313 and 366 nm. The reaction vessel should be of sufficient volume to permit removal of samples for analysis without significantly altering the volume of the reaction solution in the cell. Details for the construction of these reaction cells may be found in Calvert and Pitts (1966) and de Mayo and Shizuka (1976).

e. Temperature Control

The effect of temperature on photochemical processes in solution is usually considered to be small since these reactions occur from the electronic excited state and this process usually has small or essentially zero activation energy. However, a number of photochemical reactions have activation energies that are in the range 3-7 kcal./mole [Barltrop and Coyle (1975)]. Thus, the reaction rate may vary with temperature to a small extent. Therefore, the reaction cell should be controlled to a temperature $t_i \pm 2°C$ in the range 20 - 30°C.

3. Cell Pathlength

Zepp (1978) described an experimental method for using an isolated wavelength band to determine the effective pathlength ℓ of any cell, and in particular for small cylindrical tubes used in environmental studies. This procedure is described in Section II.D. This procedure has been used to measure the effective pathlength of the recommended Corning Glass culture tubes 13 x 100 mm; and the effective pathlength was found to be 11.2 mm [Mill et al. (1982a)]. These researchers also measured reaction tubes made from borosilicate glass stock of 12 mm o.d. and the effective pathlength was found to be 10.0 mm. If the cell pathlength was measured by this procedure, then report all the data obtained in these experiments in the Test Data Report Section of the Test Guideline including a complete description of the actinometer used. This procedure can be used to measure the effective pathlength of cylindrical cells designed for the POB apparatus; however, it is easier to measure the pathlength of these cylindrical cells directly with a precise centimeter ruler or an equivalent measuring device.

4. Solvents

Pure distilled water is used because dissolved impurities could sensitize or otherwise affect the rate of photolysis. If the half-life of an aqueous solution of test chemical in the photochemical equipment is less than 24 hours, then distilled water meeting ASTM Type II standards (or an equivalent grade) is adequate and is recommended for use.

In addition, if the half-life of an aqueous solution of test chemical in the photochemical equipment is greater than 24 hours, then the water needs to be sterile because bacteria may consume or alter the test chemical during prolonged periods of testing which may occur in the course of the rate determination. Thus, water meeting ASTM Type IIA standards, or an equivalent grade, is highly recommend to minimize biodegradation.

Furthermore, it is important that the water be saturated with air prior to preparation of the test chemical solutions to simulate environmental conditions. Air-saturated Type II water can be easily prepared by allowing the water to be equilibrated in a vessel plugged with cotton while air-saturated ASTM Type IIA water can be prepared by allowing the water to equilibrate in a vessel plugged with sterile cotton.

Reagant grade acetonitrile is recommended as the organic cosolvent with water in photochemical studies to avoid the potential effects of impurities on the rate of photolyis. Spectrograde acetonitrile or methanol is recommended for spectroscopic studies to determine the molar absorptivity of the test chemical. Overestimates of molar absorptivities may occur when spectroscopic grade solvents are not used because the impurities may absorb in the same spectral region as the test chemical. All scientists use spectroscopic grade solvents in molar absorptivity and other spectroscopic studies.

5. Sterilization

Sterilization is necessary to kill the bacteria and therefore eliminate or minimize biodegradation which could interfere with the photolysis rate determination. The presence of bacteria in either the test solutions or controls may cause biodegradation of the test substance when the photolysis experiments are carried out over a long period of time. This will introduce an error in the concentration of the test chemical. Thus, it is extremely important to use aseptic conditions in carrying out long-term photolysis experiments to minimize biodegradation. Glassware can be sterilized easily in an autoclave or by use of any other suitable non-chemical method.

6. Preparation of Test Chemical Solution

If the chemical substance is too difficult to dissolve in pure form to permit reasonable handling and analytical procedures, than a test solution may be prepared by first dissolving a chemical in reagent grade acetonitrile. The final acetonitrile concentration in the test solution should be no more than one volume percent in order to avoid solvent effects (Smith et al. 1977, 1978). Acetonitrile was chosen as a solvent as it is soluble in water, is non-polar and thus effective in dissolving many substances which are insoluble in water, it does not absorb radiation over the wavelength range of 290 to 800 nm, and it causes minimal solvent effects (i.e., minimum shifts in bands and changes in absorbance) for test substances.

7. pH Effects

The molecular structure of a chemical substance which ionizes or protonates is a function of the pH. As a result, the absorption spectrum and consequently the rate of photolysis may change with pH. The recommended procedure is to determine the uv-visible spectra under conditions in which only one species strongly predominates in solution. This can be accomplished by preparing buffered solutions containing the test chemical at pHs at least 2 pH units above the pK_a and at least 2 pH units below the pK_a. For example, the pK_a for phenol is 9.89. At a pH below 7.89, the protonated form of the phenol strongly predominates; at a pH of greater than 11.89, the phenoxide form of the phenol strongly predominates. The reaction quantum yield for ionizable compounds should also be determined in buffered solutions, where only one species strongly predominates. Thus, photolysis experiments should be carried out at the same pHs used to obtain the spectrum of each species.

Since buffers could influence the rate of photolysis, the recommended buffers for use in this Test Guideline were carefully chosen to be transparent to radiation between 290 and 800 nm and should be kept at very low concentrations to avoid buffer effects which may cause transformation of the substances by, for example, catalysis.

8. Volatile Chemical Substances

Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels. Disposable culture tubes with Teflon-lined screw caps or quartz tubes with quartz or borosilicate screw caps, Teflon-lined, are recommended. Grease should not be used since hydrophobic chemicals may adsorb to it. Volatile compounds can be conveniently studied in culture tubes equipped with gas-tight Mininert® valves. Samples can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate [Zepp (1984)]. As an alternative, the tubes can be sealed with a torch. In addition, the reaction vessels should be as completely filled as possible to prevent volatilization to any air space.

9. Control Solution

Undetected loss of a test substance through volatilization, hydrolysis, or other processes during the course of the photolysis experiment will result in the determination of erroneously large rate constants for aqueous photolysis. Therefore, for volatile chemical substances, it is important that the reaction vessels and control vessels be filled as completely as possible and sealed in order to avoid evaporative losses. To correct for possible losses, control solutions of test substance, in darkened vessels, are placed in the apparatus or alongside the apparatus and the contents of the control vessels are analyzed at the same time as the photolyzed samples (i.e., at time t). In this way, the loss of test chemical for processes other than photolysis may be determined and eliminated or accounted for in determining k_{pc}. For simplicity, if the loss of chemical in the control is small (i.e., approximately 10% or less), one can calculate a first-order loss, k_{loss}, and subtract it from

$(k_{pc})_{obs.}$ to give the corrected direct photolysis rate constant k_{pc}. If hydrolysis is found to be significant (greater than 10%), hydrolysis studies should be carried out first to define precisely the kinetics of this process [Test Guideline CG-5000].

10. Absorption Spectrum as a Criterion for Performing the Reaction Quantum Yield Experiments

The test method is applicable to all chemicals that have uv-visible absorptions in the range 290-800 nm. Thus, the uv-visible absorption spectrum of a chemical in aqueous solution will give a good indication of whether it would be useful to carry out these photolysis experiments and determine the reaction quantum yield of the test chemical. For more details, see Section II.I.

11. Actinometry

A detailed discussion of actinometry is given in Section II.C. However, some further details must be considered for the two low optical density actinometers PNAP/PYR and PNA/PYR discussed in Sections II.C.1.a and b, respectively. In order to carry out Procedure One, it is necessary to choose the appropriate actinometer and to adjust the concentration of pyridine so that the rate constant of the actinometer approximately equals the rate constant of the test chemical. The rate constant of the actinometer is a function of the molar concentration of pyridine [PYR], the intensity of light incident on the actinometer (I_λ), and the molar absorptivity of the actinometer $\varepsilon_{\lambda a}$. The pertinent equations for the low optical density actinometer are equations 41 and 20. For Procedure One, it is necessary to derive equations under the condition that the rate constant of the actinometer equals the rate constant of the test chemical; that is,

$$k_{pa} = k_{pc} \quad . \tag{57}$$

Substituting equation 41 in equation 57 yields

$$k_{pc} = 2.303\phi_a I_\lambda \ell \varepsilon_{\lambda a} \; . \tag{58}$$

Substituting equation 41 in equation 20 yields

$$(t_{1/2})_a = 3.01\mathrm{x}10^{-1} [\phi_a I_\lambda \ell \varepsilon_{\lambda a}]^{-1} \; . \tag{59}$$

These equations are perfectly general and are applicable for use in a POB or PMGRR using any light source filtered to yield a monochromatic wavelength band λ. Equations will be derived for a typical 450 watt medium pressure mercury lamp in an Ace-type or a Moses-type PMGRR. The pertinent information has been taken from the paper by Dulin and Mill (1982), the data from Sections II.C.1.a and b, and from Mill (1984).

a. p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR) in an Ace-type PMGRR.

For the PNAP/PYR actinometer, the relationship of the reaction quantum yield (ϕ_a) as a function of the molar concentration of pyridine [PYR] is given by equation 30

$$\phi_a = 0.0169[\mathrm{PYR}] \quad . \tag{30}$$

For the 450 watt medium pressure mercury lamp in an Ace-type PMGRR used by Mill and Dulin (1982) the approximate average light intensity at 313 nm and 366 nm was: $I_{(313)} = 3.0 \times 10^{-6}$ einsteins sec^{-1} L^{-1}; $I_{(366)} = 7.5 \times 10^{-6}$ einsteins sec^{-1} L^{-1}. Conversion of these intensities to hours yields $I_{(313)} = 1.08 \times 10^{-2}$ einsteins $\mathrm{hr.}^{-1}$ L^{-1} and $I_{(366)} = 2.70 \times 10^{-2}$ einsteins $\mathrm{hr.}^{-1}$ L^{-1}. For the cells used by Dulin and Mill (1982), $\ell = 1.0$ [Mill (1984)]. The molar absorptivities of PNAP at 313 and 366 nm are: $\varepsilon_{313a} = 2056\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ and $\varepsilon_{366a} = 160\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ (Section II.C.1.a).

At 313 nm, the following relationships can be calculated. Substituting equation 30 in equation 58 and using the above pertinent data:

$$k_{pc} = 2.303(1.69 \times 10^{-2}[\mathrm{PYR}])(1.08 \times 10^{-2})(1.0)(2.056 \times 10^{3})$$

$$k_{pc} = 0.864[\mathrm{PYR}] \tag{60}$$

$$[PYR] = 1.16\ k_{pc} \quad , \qquad (61)$$

where k_{pc} is in the units $(hours)^{-1}$ and [PYR] is in the units of molar concentration. Equation 61 corresponds to equation 18 listed in the Test Guideline. Furthermore, since $k_{pa} = k_{pc}$ (equation 57), equation 60 becomes

$$k_{pa} = 0.864[PYR] \quad ; \qquad (62)$$

and substituting this result in equation 20 yields

$$(t_{1/2})_a = 0.802/[PYR] \quad , \qquad (63)$$

where $(t_{1/2})_a$ is the half-life of the actinometer in hours. The half-life of the actinometer can range from 16 hours at [PYR] = 0.05 M to 33 days at $[PYR] = 10^{-3}$ M. Thus, this actinometer is suitable for use with test chemicals having half-lives > 12 hours to several weeks.

The above calculation can be repeated at 366nm and the results are:

$$[PYR] = 5.95\ k_{pc} \quad , \qquad (64)$$

where k_{pc} is in the units of $(hours)^{-1}$ and [PYR] is in the units of molar concentration. Equation 64 corresponds to equation 19 listed in the Test Guideline. The half-life of the actinometer in hours is given by the equation

$$(t_{1/2})_a = 4.13/[PYR] \quad . \qquad (65)$$

Using the molar absorptivity data for PNAP at 313 and 366 nm, the absorbance A_{313a} and A_{366a} is less than 0.02 in a 1.00 cm absorption cell containing a PNAP solution of concentration $< 1 \times 10^{-5}$ M. The recommended tubes used for these photolysis experiments are approximately 1 cm. Hence, PNAP solutions of concentration $< 1 \times 10^{-5}$ M have absorbance less than 0.02 in the recommended tubes and thus correspond to low optical density. Thus, PNAP solutions of concentration $<$ 1×10^{-5} M have been recommended for use in this Test Guideline.

b. p-Nitroanisole-Pyridine Actinometer (PNA/PYR) in an Ace-Type PMGRR

The above calculations can be repeated as described above using equation 31 for the PNA/PYR actinometer.

$$\phi_a = 0.437[PYR] + 0.000282 \quad . \qquad (31)$$

$I_{(313)} = 1.08 \times 10^{-2}$ einsteins hr.$^{-1}$ L^{-1} and $I_{(366)} = 2.70 \times 10^{-2}$ einsteins hr.$^{-1}$ L^{-1}; $\ell = 1.0$; $\epsilon_{313a} = 1.03 \times 10^4$ M^{-1} cm^{-1} and $\epsilon_{366a} = 1.99 \times 10^3$ M^{-1} cm^{-1} (Section II.C.1.b).

Thus, at 313 nm:

$$[PYR] = 8.93 \times 10^{-3}(k_{pc} - 0.0722), \qquad (66)$$

where k_{pc} is in the units $(\text{hours})^{-1}$ and [PYR] is in the units of molar concentration. Equation 66 corresponds to equation 20 listed in the Test Guideline. The half-life of the actinometer in hours is given by the equation

$$(t_{1/2})_a = (162[\text{PYR}] + 0.104)^{-1} \quad . \tag{67}$$

The half-life of the actinometer can range from 15 minutes at [PYR] = 0.02M to 8.3 hours at $[\text{PYR}] = 10^{-4}$M. Thus, this actinometer is suitable for use with test chemicals having half-lives of > 15 minutes to approximately 12 hours:

At 366 nm:

$$[\text{PYR}] = 1.85 \times 10^{-2}(k_{pc} - 0.0349) \quad , \tag{68}$$

where k_{pc} is in the units of $(\text{hour})^{-1}$ and [PYR] is in the units of molar concentration. Equation 68 corresponds to equation 21 listed in the Test Guideline. The half-life of the actinometer is given by the equation

$$(t_{1/2})_a = (78.1[\text{PYR}] + 0.0504)^{-1} \quad . \tag{69}$$

Using the molar absorptivity of PNA at 366 nm, the absorbance A_{366a} is less than 0.02 in a 1.00 cm absorption cell containing a PNA solution $< 1 \times 10^{-5}$ M . The recommended tubes used for these photolysis experiments are approximately 1 cm.

Hence, PNA solutions of concentration $< 1 \times 10^{-5}$ M have absorbance less than 0.02 in the recommended tubes and this corresponds to low optical density at 366 nm. However, at 313 nm, the molar absorptivity is large and A_{313a} is less than 0.02 in a 1.00 cm absorption cell containing a PNA solution of concentration $< 0.2 \times 10^{-5}$ M. PNA is difficult to analyze at a concentration significantly below 0.2×10^{-5} M. As a compromise, the allowable absorbance has been raised to 0.04 and the concentration of PNA in a 1.00 cm cell is 0.4×10^{-5} M. Thus, the concentration of PNA has been set at 0.4×10^{-5} M for photolysis experiments at 313 nm in the recommended tubes. PNA can be analyzed in this concentration range and only a small error (< 10%) has been introduced in the method.

c. The PNAP/PYR and PNA/PYR Actinometers in a Moses-Type PMGRR

Test Guideline CG-6010 has been designed for use with an Ace-type PMGRR which is commercially available. However, if a Moses-type PMGRR (Section II.J.2.a) is available, the discussions given in Sections II.J.11.a and b have to be modified. As indicated in Section II.J.2.a, the Ace-type PMGRR is designed so that the entire reaction tubes are irradiated while the Moses-type PMGRR is designed with windows, which act as slits, and as a result irradiation only occurs in a narrow portion of the reaction cells. Thus, the light intensity is reduced in a Moses-type PMGRR thereby reducing the reaction rate of a chemical. Since the reaction rate of the chemical is reduced, the corresponding half-life is increased. Mill (1984) has indicated that his Ace-type PMGRR had an effective aperture of 5.35 cm^2 while his Moses-type PMGRR (which he had specially built) had an aperture of 1.21 cm^2. Since $t_{1/2}$ is proportional to the reciprocal of the rate constant k_p and k_p is proportional to the light intensity, $t_{1/2}$ is proportional to the reciprocal of the light intensity. Furthermore, since the light intensity is proportional to the aperture (A_p in cm^2), $t_{1/2}$ is proportional to the reciprocal of the aperture.

Using the subscript notation Ace to represent the Ace-type PMGRR and the subscript notation Test to represent the Moses-type PMGRR, then for a test chemical (c), one can write

$$\frac{(t_{1/2})_{c,\ Ace}}{(t_{1/2})_{c,\ Test}} \propto \frac{[1/(A_p)_{Ace}]}{[1/(A_p)_{Test}]} \qquad ,$$

assuming that the same reaction cells are placed in both types of PMGRR (and thus have the same pathlength).

$$[(t_{1/2})_{c,Ace}/(t_{1/2})_{c,Test}] = (A_p)_{Test}/(A_p)_{Ace}$$

$$(t_{1/2})_{c,Test} = (t_{1/2})_{c,Ace}[(A_p)_{Ace}/(A_p)_{Test}] \quad . \qquad (69)$$

From the previous discussions, the criteria for the choice of actinometer were: if $(t_{1/2})_c$ is less than 12 hours, use the PNA/PYR actinometer; if $(t_{1/2})_c$ is greater than 12 hours, then use the PNAP/PYR actinometer. Thus, $(t_{1/2})_{c,Ace} = 12$ hours. Since $(A_p)_{Ace} = 5.35\ cm^2$, then equation 69 becomes

$$(t_{1/2})_{c,Test} = 64/(A_p)_{Test} \quad , \qquad (70)$$

where $(t_{1/2})_{c,Test}$ is in hours and A_p is the aperture in cm^2. Therefore, if a Moses-type PMGRR is used in this Test Guideline (CG-6010), the aperture (A_p) must be measured and substituted in equation 70 and the test criteria will be: if $(t_{1/2})_{c,Test} < 64/(A_p)_{Test}$, then use the PNA/PYR actinometer; and if $(t_{1/2})_{c,Test} > 64/(A_p)_{Test}$, then use the PNAP/PYR actinometer.

Using the same approach as described above, equations 61 and 64 (for the PNAP/PYR actinometer) and equations 66 and 68 (for the PNA/PYR actinometer) can be modified as follows. Based on the above discussions, it can be readily shown that

$$\frac{(k_p)_{c,Test}}{(k_p)_{c,Ace}} \propto \frac{(A_p)_{Test}}{(A_p)_{Ace}}$$

$$(k_p)_{c,Ace} = (k_p)_{c,Test}\ [(A_p)_{Ace}/(A_p)_{Test}] \quad . \qquad (71)$$

For the Ace-type PMGRR, the aperture is $5.35\ cm^2$ [Mill (1984)]. Thus, equation 71 becomes

$$(k_p)_{c,Ace} = 5.35(k_p)_{c,Test}/(A_p)_{Test} \quad , \qquad (72)$$

where the rate constants are in the units of $hours^{-1}$ and $(A_p)_{Test}$ is the aperture of the Moses-type PMGRR in cm^2.

For the PNAP/PYR actinometer, equations 61 and 64 define the pyridine concentration needed to adjust the rate constant of the actinometer to equal the rate constant of the test chemical in the Ace-type PMGRR at 313 and 366 nm, respectively. The corresponding equations for the Moses-type PMGRR can be obtained by substituting equation 72 in equations 61 and 64. The results are:

at 313 nm

$$[PYR] = 6.21(k_p)_{c,Test}/(A_p)_{Test} \quad ; \qquad (73)$$

at 366 nm

$$[PYR] = 31.8(k_p)_{c,Test}/(A_p)_{Test} \quad , \qquad (74)$$

where $(k_p)_{c,Test}$ is in the units of $hour^{-1}$, $(A_p)_{Test}$ is the aperture of the Moses-type PMGRR in the units of cm^2, and [PYR] is in the units of molar concentration.

Following the same procedure as above for the PNA/PYR actinometer, equations 66 and 68 can be modified using equation 72. The results are:

at 313 nm

$$[\mathrm{PYR}] = 8.93 \times 10^{-3} \left\{ [5.35(k_p)_{c,\mathrm{Test}}/(A_p)_{\mathrm{Test}}] - 0.0722 \right\} ; \qquad (75)$$

at 366 nm

$$[\mathrm{PYR}] = 1.85 \times 10^{-2} \left\{ [5.35(k_p)_{c,\mathrm{Test}}/(A_p)_{\mathrm{Test}}] - 0.0349 \right\} , \qquad (76)$$

where $(k_p)_{c,\mathrm{Test}}$ is in the units of hour^{-1}, $(A_p)_{\mathrm{Test}}$ is the aperture of the Moses-type PMGRR in the units of cm^2 and [PYR] is in the units of molar concentration.

12. Chemical Analysis of Solutions

a. Chemical Analysis of Test Chemical Solutions

The analytical techniques employed in the determination of the concentration of the test substances are left to selection by the tester. This is in recognition of the many different techniques available and the practical advantage of being able to make particular use of one of the properties of the substance; e.g., the NMR or UV spectrum of the substance, or its chromatographic behavior. Analytical techniques that permit the determination of the test compound to the exclusion of impurities or photolysis reaction products are recommended to the extent practicable. Therefore, chromatographic techniques are particularly desirable. Whenever practicable, an analytical procedure should be used which has a precision of ± 5% or better. The specific technique which is utilized should be completely described.

b. Chemical Analysis of p-Nitroacetophenone (PNAP)

The p-nitroacetophenone (PNAP) in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 2.5% acetic acid, 50% acetonitrile, and 47.5% water which is passed through the column at a flow rate of 2 mL/minute. This analytical procedure was specifically developed to analyze for PNAP, especially at high pyridine concentrations (i.e., for pyridine concentrations up to approximately 0.2 M) [Winterle (1984)].

c. Chemical Analysis of p-Nitroanisole (PNA)

The p-nitroanisole (PNA) in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 50% acetonitrile and 50% water which is passed through the column at a flow rate of 2 mL/minute. This analytical procedure was developed by Dulin and Mill (1982).

d. Chemical Analysis of Ferrous Ion in the Ferrioxalate Actinometer

The molar concentration of Fe^{2+} formed in the photolysis of the ferrioxalate actinometer is measured spectrophotometrically via the formation of a red phenanthroline complex and determining the absorbance at 510 nm. This is the standard procedure for determining the molar concentration of Fe^{2+} formed in the photolysis of the ferrioxalate actinometer. The procedure described by Murov (1973) is excellent and has been recommended. The procedure has been modified slightly by using the concentration of ferrioxalate at 0.15 M. As a result, the irradiated solution has to be diluted 100-fold prior to analysis for Fe^{2+}, which must not be allowed to exceed 0.005 M [Zepp (1984)].

III. REFERENCES

AA. 1982. Astronomical Almanac.

Barltrop JA and Coyle JD. 1975. Excited states in organic chemistry. John Wiley and Sons, Inc., New York, New York.

Balzani V and Carassiti V. 1970. Photochemistry of coordination compounds. Academic Press, New York, New York.

Benson SW. 1976. Thermochemical kinetics. Second Edition. Wiley-Interscience, New York, New York.

Calvert JG and Pitts JN, Jr. 1966. Photochemistry. John Wiley and Sons, Inc., New York, New York.

de Mayo P and Shizuka P. 1976. Measurement of reaction quantum yields. "Creation and Detection of Excited States," Vol. 4, WR Ware, Ed. Marcel Dekker, Inc., New York, New York.

Demerjian KL, Schere KL, and Peterson JT. 1980. Theoretical estimates of actinic (spherically integrated) flux and photolysis rate constants of atmospheric species in the lower troposphere. Adv Sci and Tech 10:389.

Dulin D and Mill T. 1982. Development and application of solar actinometers. Environ Sci and Tech 16:815.

Hatchard CG and Parker CA. 1956. A new sensitive chemical actinometer II. Potassium ferrioxylate as a standard chemical actinometer. Proc Royal Soc of London A 235:518.

Leighton PA. 1961. Photochemistry of air pollution. Academic Press, New York, New York.

Mill T, Davenport JE, Dulin DE, Mabey WR, and Bawol R. 1981. Evaluation and optimization of photolysis screening protocols. U.S. Environmental Protection Agency. EPA-560/5-81-003.

Mill T, Mabey WR, Hendry DG, Winterle J, Davenport J, Barich V, Dulin D, and Tse D. 1982. Design and validation of screening and detailed methods for environmental process. EPA- .

Mill T, Mabey WR, Bomberger DC, Chou T-W, Hendry DG, and Smith JH. 1982a. Laboratory protocols for evaluating the fate of organic chemicals in air and water. EPA-600/3-82-022.

Mill T. 1984. Private communication. SRI International, Menlo Park, California.

Moses FG, Liu RSH, and Monroe BM. 1969. The "merry-go-round" quantum yield apparatus. Mol Photochem 1(2):245.

Murov SL. 1973. Handbook of photochemistry. Marcel Dekker, Inc., New York, New York.

Parker CA. 1953. A new sensitive chemical actinometer I. Some details with potassium ferrioxylate. Proc Royal Soc of London A 220:104.

Peterson JT. 1976. Calculated actinic fluxes (290-700nm) for air pollution photochemistry applications. EPA-600/4-76-0025.

Pierce BO. 1929. A short table of integrals. Ginn and Company, New York, New York.

Pitts JN, Wan JKS, and Schuck EA. 1964. An o-nitrobenzaldehyde actinometer and its application to a kinetic study of the photoreduction of benzophenone by benzhydrol in a pressed potassium bromide disk. J Am Chem Soc 86:3606.

Smith JH, Mabey WR, Bohonos N, Holt BR, Lee SS, Chou T-W, Bomberger DC, Mill T. 1977. Environmental pathways of selected chemicals in freshwater systems. Part I. Background and experimental procedures. U.S. Environmental Protection Agency, Athens, GA. EPA 600/7-77-113.

Smith JH, Mabey WR, Bohonos N, Holts BR, Lee SS, Chou T-W, Bomberger DC, Mill T. 1978. Environmental pathway of selected chemicals in freshwater systems. Part II. Laboratory studies. U.S. Environmental Protection Agency, Athens, GA. EPA 600/7-78-074.

Vesley CF. 1971. Complications in measuring quantum yields using cylindrical cells. Mol Photochem 3(2):193.

Wegner EE and Adamson AW. 1966. Photochemistry of complex ions III. Absolute quantum yields for the photolysis of some aqueous chromium (III) complexes. Chemical actinometry in the long wavelength visible region. J Am Chem Soc 88:394(1966).

Winterle J. 1984. Private communication. SRI International, Menlo Park, California.

Zepp RG and Cline DM. 1977. Rates of direct photolysis in aquatic environment. Environ Sci and Technol 11:359-366.

Zepp RG. 1978. Quantum yields for reaction of pollutants in dilute solution. Environ Sci and Technol 12:327.

Zepp RG. 1982. Experimental approaches to environmental photochemistry. The handbook of environmental chemistry. O. Hutzinger, Editor. Springer-Verlag, New York, New York.

Zepp RG. 1984. Private communication. Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.

IV. APPENDIX: GLOSSARY OF IMPORTANT SYMBOLS

λ	Wavelength λ .
A_λ	Absorbance at wavelength λ.
$F_{s\lambda}$	Fraction of light absorbed by the system at wavelength λ.
$F_{c\lambda}$	Fraction of light absorbed by a chemical (c) at wavelength λ.
$\varepsilon_{\lambda c}$	Molar absorptivity of a chemical (c) at wavelength λ.
$\varepsilon_{\lambda a}$	Molar absorptivity of an actinometer (a) at wavelength λ.
α_λ	Absorption (or attenuation) coefficient of water at wavelength λ.
ℓ	The light pathlength; the distance traveled by a beam of light passing through the system.
ϕ_a	Reaction quantum yield of an aqueous solution of actinometer (a).
ϕ_c	Reaction quantum yield of an aqueous solution of chemical (c).
ϕ_E	Sunlight reaction quantum yield of a chemical (c) in a water body in the environment.
$-d[C]/dt$	Direct photolysis rate of chemical (c).
k_{pE}	Direct photolysis sunlight rate constant in a water body in the environment.
$(k_{pE})_{max.}$	Maximum direct photolysis rate constant in a water body in the environment.
k_{pc}	Direct photolysis rate constant of chemical (c) in water measured in the laboratory.
k_{pa}	Direct photolysis rate constant of actinometer (a) in water measured in the laboratory.
$k_{a\lambda}$	Specific light absorption of a photoreactive chemical at low concentration and at wavelength λ.
k_a	Specific light absorption rate constant integrated over all wavelengths absorbed by the chemical.

$(t_{1/2})_c$	Half-life of a chemical (c) in water.
$(t_{1/2})_a$	Half-life of an actinometer (a) in water.
$t_{1/2E}$	Half-life of a chemical in a water body in sunlight in the environment.
$(t_{1/2E})_{min.}$	The minimum sunlight half-life of a chemical in a water body in sunlight in the environment.
$I_{o\lambda}$	The number of photons (or einsteins) of light of wavelength in the system per cm^2 per second.
I_λ	The number of einsteins of light of wavelength in the system per liter per hour (or second).
L_λ	Solar irradiance in a water body at shallow depths in the units milli einsteins per cm^2 per second.
PYR	Pyridine.
[PYR]	Molar concentration of pyridine.
PNAP	p-Nitroacetophenone.
PNA	p-Nitroanisole.
o-NB	o-Nitrobenzaldehyde
PNAP/PYR	p-Nitroacetophenone-pyridine actinometer.
PNA/PYR	p-Nitroanisole-pyridine actinometer.
POB	Photochemical optical bench.
PMGRR	Photochemical "Merry-Go-Round" Reactor.
A_p	Aperture in the PMGRR.

$(t_{1/2})_c$	Half-life of a chemical (c) in water.
$(t_{1/2})_a$	Half-life of an actinometer (a) in water.
$t_{1/2E}$	Half-life of a chemical in a water body in sunlight in the environment.
$(t_{1/2E})_{min.}$	The minimum sunlight half-life of a chemical in a water body in sunlight in the environment.
$I_{o\lambda}$	The number of photons (or einsteins) of light of wavelength λ in the system per cm^2 per second.
I_λ	The number of einsteins of light of wavelength λ in the system per liter per hour (or second).
L_λ	Solar irradiance in a water body at shallow depths in the units milli einsteins per cm^2 per second.
PYR	Pyridine.
[PYR]	Molar concentration of pyridine.
PNAP	p-Nitroacetophenone.
PNA	p-Nitroanisole.
o-NB	o-Nitrobenzaldehyde
PNAP/PYR	p-Nitroacetophenone-pyridine actinometer.
PNA/PYR	p-Nitroanisole-pyridine actinometer.
POB	Photochemical optical bench.
PMGRR	Photochemical "Merry-Go-Round" Reactor.
A_p	Aperture in the PMGRR.